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(54) Title: NUCLEIC ACID ENCODING NEUROPEPTIDE Y/PEPTIDE YY (Y2) RECEPTORS AND USES THEREOF

(57) Abstract

This invention provides isolated nucleic acid molecules encoding Y2 receptors, an isolated, purified Y2 receptor protein, vectors comprising isolated nucleic acid molecules encoding Y2 receptors, mammalian, insect, bacterial and yeast cells comprising such vectors, antibodies directed to the Y2 receptors, nucleic acid probes useful for detecting nucleic acid encoding Y2 receptors, antisense oligonucleotides complementary to unique sequences of a nucleic acid molecule which encodes a Y2 receptor, pharmaceutical compounds related to the Y2 receptors, and nonhuman transgenic animals which express nucleic acid encoding a normal or mutant Y2 receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and methods of treatment involving Y2 receptors.

NUCLEIC ACID ENCODING NEUROPEPTIDE Y/PEPTIDE YY (Y2) RECEPTORS AND USES THEREOF

Background of the Invention

5   This application is a continuation-in-part of U.S. Serial No. 08/192,288, filed February 3, 1994, the contents of which are hereby incorporated by reference into the subject application.

10   Throughout this application, various publications are referenced in parenthesis by number. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosure of these publications is hereby incorporated

15   by reference into this application to describe more fully the art to which this invention pertains.

    Neuropeptides are small peptides originating from large precursor proteins synthesized by peptidergic neurons and

20   endocrine/paracrine cells. They hold promise for treatment of neurological, psychiatric, and endocrine disorders (46). Often the precursors contain multiple biologically active peptides. There is great diversity of neuropeptides in the brain caused by alternative

25   splicing of primary gene transcripts and differential precursor processing. The neuropeptide receptors serve to discriminate between ligands and to activate the appropriate signals.

30   Neuropeptide Y (NPY), a 36-amino acid peptide, is the most abundant neuropeptide to be identified in mammalian brain. NPY is an important regulator in both the central and peripheral nervous systems (47) and influences a diverse range of physiological parameters, including

35   effects on psychomotor activity, food intake, central endocrine secretion, and vasoactivity in the

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cardiovascular system. High concentrations of NPY are found in the sympathetic nerves supplying the coronary, cerebral, and renal vasculature and have contributed to vasoconstriction. NPY binding sites have been identified

5 in a variety of tissues, including spleen (48), intestinal membranes, brain (49), aortic smooth muscle (50), kidney, testis, and placenta (2). In addition, binding sites have been reported in a number of rat and human cell lines (e.g. Y1 in SK-N-MC, MC-IXC, CHP-212,

10 and PC12 cells; Y2 in SK-N-Be(2), CHP-234, and SMS-MSN) (51,5).

Neuropeptide Y (NPY) receptor pharmacology is currently defined by structure activity relationships within the

15 pancreatic polypeptide family (1, 2). This family includes NPY, which is synthesized primarily in neurons; peptide YY (PYY), which is synthesized primarily by endocrine cells in the gut; and pancreatic polypeptide (PP), which is synthesized primarily by endocrine cells

20 in the pancreas. These 36 amino acid peptides have a compact helical structure involving a "PP-fold" in the middle of the peptide. Specific features include a polyproline helix in residues 1 through 8, a β-turn in residues 9 through 14, an α-helix in residues 15 through

25 30, an outward-projecting C-terminus in residues 30 through 36, and a carboxyl terminal amide which appears to be critical for biological activity (3). The peptides have been used to define at least four receptor subtypes known as Y1, Y2, Y3, and PP. Y1 receptor recognition by

30 NPY involves both N- and C-terminal regions of the peptide; exchange of Gln[34] with Pro[34] is fairly well tolerated (3, 4, 5). Y2 receptor recognition by NPY depends primarily upon the four C-terminal residues of the peptide (Arg[33]- Gln[34]-Arg[35]- Tyr[36]-NH$_2$) preceded by an

35 amphipathic α-helix (3, 6, 7); exchange of Gln[34] with Pro[34] is not well tolerated (4, 5). Y3 receptor recognition is characterized by a strong preference for NPY over PYY

(8). Exchange of Gln^{34} in NPY with Pro^{34} is reasonably well tolerated by the Y3 receptor but PP, which also contains Pro^{34}, does not bind well (8). The PP receptor is reported to bind tightly to PP, less so to

5 $[Leu^{31},Pro^{34}]$NPY, and even less so to NPY (3, 9). The only NPY receptor which has been cloned to date is the Y1 receptor gene, from mouse (12), rat (52), and human (10). One of the key pharmacological features which distinguish Y1 and Y2 is the fact that the Y1 receptor (and not the

10 Y2 receptor) responds to an analog of NPY modified at residues 31 and 34 ([Leu31,Pro34]NPY), whereas the Y2 receptor (and not the Y1 receptor) has high affinity for the NPY peptide carboxyl-terminal fragment NPY-(13-36) (53,4).

15

Receptor genes for the other two structurally related peptides, peptide YY (PYY) and pancreatic polypeptide (PP), also have not been cloned. Peptide YY occurs mainly in endocrine cells in the lower gastrointestinal

20 tract (54). Receptors for PYY were first described in the rat small intestine (55). This receptor has been defined as PYY-preferring because it displays a 5-10 fold higher affinity for PYY than for NPY (55,56). Recently, a cell line, PKSV-PCT, derived from the proximal tubules

25 of kidneys, has been described to express receptors for PYY (57).

In the last few years only the rat and human Y1 cDNAs have been cloned (10, 11). This success was based on

30 identifying the randomly cloned FC5 "orphan receptor" (12). We now report the isolation by expression cloning of a human hippocampal Y2 cDNA clone and two rat Y2 clones and their pharmacological characterization.

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Summary of the Invention

This invention provides an isolated nucleic acid molecule encoding a Y2 receptor.

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This invention also provides an isolated protein which is a Y2 receptor.

This invention provides a vector comprising nucleic acid
10   encoding a Y2 receptor.

This invention also provides vectors such as plasmids comprising nucleic acid encoding a Y2 receptor, adapted for expression in a bacterial cell, a yeast cell, an
15   insect cell or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the nucleic acid in the bacterial, yeast, insect or mammalian cells operatively linked to the nucleic acid encoding the Y2 receptor as to permit expression thereof.
20
This invention provides a cell transfected with and expressing nucleic acid encoding a Y2 receptor.

This invention provides a nucleic acid probe comprising
25   a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a Y2 receptor.

30   This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing with any sequences of an mRNA molecule which encodes a Y2 receptor so as to prevent translation of the mRNA molecule.
35
This invention provides an antibody directed to a Y2 receptor.

5

This invention provides a transgenic nonhuman mammal expressing nucleic acid encoding a Y2 receptor. This invention further provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA

5   encoding a Y2 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a Y2 receptor and which hybridizes to mRNA encoding a Y2 receptor thereby reducing its translation.

10 This invention further provides a transgenic nonhuman mammal comprising a homologous recombination knockout of the native Y2 receptor.

This invention provides a method for determining whether
15 a ligand can bind specifically to a Y2 receptor which comprises contacting a cell transfected with and expressing nucleic acid encoding the Y2 receptor with the ligand under conditions permitting binding of ligands to such receptor, and detecting the presence of any such
20 ligand bound to the Y2 receptor, thereby determining whether the ligand binds specifically to a Y2 receptor.

This invention also provides a method for determining whether a ligand is a Y2 receptor agonist which comprises
25 contacting a cell transfected with and expressing nucleic acid encoding the Y2 receptor with the ligand under conditions permitting the activation of a functional Y2 receptor response from the cell, and detecting, by means of a bioassay, such as a second messenger assay, an
30 increase in Y2 receptor activity, thereby determining whether the ligand is a Y2 receptor agonist.

This invention further provides a method for determining whether a ligand is a Y2 receptor antagonist which
35 comprises contacting a cell transfected with and expressing nucleic acid encoding the Y2 receptor with the ligand in the presence of a known Y2 receptor agonist,

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such as NPY, under conditions permitting the activation
of a functional Y2 receptor response, and detecting, by
means of a bioassay, such as a second messenger assay, a
decrease in Y2 receptor activity, thereby determining
5   whether the ligand is a Y2 receptor antagonist.

This invention further provides a method of screening
drugs to identify drugs which specifically bind to a Y2
receptor on the surface of a cell which comprises
10 contacting a cell transfected with and expressing nucleic
acid encoding the Y2 receptor with a plurality of drugs
under conditions permitting binding of drugs to the Y2
receptor, and determining those drugs which bind to the
Y2 receptor, thereby identifying drugs which specifically
15 bind to a Y2 receptor.

This invention also provides a method of screening drugs
to identify drugs which act as agonists of a Y2 receptor
on the surface of a cell which comprises contacting a
20 cell transfected with and expressing nucleic acid
encoding the Y2 receptor with a plurality of drugs under
conditions permitting the activation of a functional Y2
receptor response, and determining those drugs which
activate the Y2 receptor, using a bioassay, such as a
25 second messenger assay, thereby identifying drugs which
act as Y2 receptor agonists.

This invention also provides a method of screening drugs
to identify drugs which act as antagonists of a Y2
30 receptor on the surface of a cell which comprises
contacting a cell transfected with and expressing nucleic
acid encoding the Y2 receptor with a plurality of drugs
in the presence of a known Y2 receptor agonist, such as
NPY, under conditions permitting the activation of a
35 functional Y2 receptor response, and determining those
drugs which inhibit the activation of the Y2 receptor,
using a bioassay, such as a second messenger assay,

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thereby identifying drugs which act as Y2 receptor
antagonists.

This invention also provides a method of detecting
5 expression of a Y2 receptor by a cell by detecting the
presence of mRNA coding for the Y2 receptor which
comprises obtaining total mRNA from the cell and
contacting the mRNA so obtained with a nucleic acid probe
comprising a nucleic acid molecule of at least 15
10 nucleotides capable of specifically hybridizing with a
unique sequence included within the sequence of a nucleic
acid molecule encoding the Y2 receptor under hybridizing
conditions, and detecting the presence of mRNA hybridized
to the probe, thereby detecting the expression of a Y2
15 receptor by the cell.

This invention provides a method of determining the
physiological effects of expressing varying levels of Y2
receptors which comprises producing a transgenic nonhuman
20 mammal expressing nucleic acid encoding a Y2 receptor
whose levels of Y2 receptor expression are varied by use
of an inducible promoter which regulates Y2 receptor
expression.

25 This invention also provides a method of determining the
physiological effects of expressing varying levels of Y2
receptors which comprises producing a panel of transgenic
nonhuman animals each expressing nucleic acid encoding a
Y2 receptor expressing nucleic acid and expressing a
30 different amount of Y2 receptor.

This invention provides a method for diagnosing a
predisposition to a disorder associated with the activity
of a specific Y2 receptor allele which comprises: a.
35 obtaining nucleic acid of subjects suffering from the
disorder; b. performing a restriction digest of the
nucleic acid with a panel of restriction enzymes; c.

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electrophoretically separating the resulting nucleic acid
fragments on a sizing gel; d. contacting the resulting
gel with a nucleic acid probe capable of specifically
hybridizing to nucleic acid encoding a Y2 receptor and
5   labeled with a detectable marker; e. detecting labeled
bands which have hybridized to the nucleic acid encoding
a Y2 receptor labelled with a detectable marker to create
a unique band pattern specific to the nucleic acid of
subjects suffering from the disorder; f. preparing
10   nucleic acid obtained for diagnosis by steps a-e; and g.
comparing the unique band pattern specific to the nucleic
acid of subjects suffering from the disorder from step e
and the nucleic acid obtained for diagnosis from step f
to determine whether the patterns are the same or
15   different and to diagnose thereby predisposition to the
disorder if the patterns are the same.

This invention provides a method of preparing an
isolated, purified Y2 receptor which comprises
20   constructing a vector adapted for expression in a cell
which comprises the regulatory elements necessary for the
expression of nucleic acid in the cell operatively linked
to the nucleic acid encoding a Y2 receptor as to permit
expression thereof, wherein the cell is selected from the
25   group consisting of bacterial cells, yeast cells, insect
cells and mammalian cells; inserting the vector of the
previous step in a suitable host cell; incubating the
cells under conditions allowing the expression of a Y2
receptor; recovering the receptor so produced and
30   purifying the receptor so recovered.

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Brief Description of the Drawings

Figure 1

5   Nucleotide sequence of the human hippocampal Y2 cDNA
    clone (SEQ. I.D. No. 1). Initiation and stop codon are
    indicated in bold. Only partial 5' and 3' untranslated
    sequences are shown.

Figure 2

10 Deduced amino acid sequence of the human hippocampal Y2
    cDNA clone encoded by the nucleotide sequence in Figure
    1 (SEQ. I.D. No. 2).

Figure 3-1 through Figure 3-4

15 Comparison of coding nucleotide sequences between human
    hippocampal Y2 (top row) and Y1 human cDNA clones (bottom
    row) (48.5% nucleotide identity).

Figure 4-1 and Figure 4-2

20 Comparison of amino acid sequences between hippocampal Y2
    (top row) and Y1 human cDNA clones (bottom row). (31%
    overall identity and 41% in the transmembrane domains).

Figure 5A

25 Equilibrium binding of ^{125}I-PYY to membranes from COS-7
    cells transiently expressing CG-13 (•) and human Y1 (o)
    receptors. Membranes were incubated with ^{125}I-PYY for the
    times indicated, in the presence or absence of 100 nM
30 human NPY. Specific binding, B, was plotted against
    time, t, to obtain the maximum number of equilibrium
    binding sites, B_1 and B_2, and observed association rates,
    K_{obs1} and K_{obs2}, according to the equation, $B = B_1 * (1 - e^{-(kobs1 * t)}) + B_2 * (1 - e^{-(kobs2 * t)})$. Binding is shown as the
35 percentage of total equilibrium binding, $B_1 + B_2$,
    determined by nonlinear regression analysis. Data are
    representative of three independent experiments, with

each point measured in triplicate.

Figure 5B

Equilibrium binding of ^{125}I-PYY to membranes from COS-7
5   cells transiently expressing CG-13 ($\bullet$) and human Y1 (o)
receptors using the same conditions as in Figure 5A
except for a prolonged time course of up to 180 minutes.

Figure 6

10 Saturable equilibrium binding of ^{125}I-PYY to membranes from
COS-7 cells transiently expressing CG-13 receptors.
Membranes were incubated with ^{125}I-PYY ranging in
concentration from 0.003 nM to 2 nM, in the presence or
absence of 100 nM human NPY. Specific binding, B, was
15 plotted against the free ^{125}I-PYY concentration, [L], to
obtain the maximum number of saturable binding sites, B_{max},
and the ^{125}I-PYY equilibrium dissociation constant, K_d,
according to the binding isotherm, $B = B_{max}[L]/([L] + K_d)$.
Specific binding is shown. Data are representative of
20 three independent experiments, with each point measured
in triplicate.

Figure 7A

Competitive displacement of ^{125}I-PYY on membranes from COS-
25 7 cells transiently expressing Human Y1 receptors.
Membranes were incubated with ^{125}I-PYY and increasing
concentrations of peptide competitors. IC_{50} values
corresponding to 50% displacement were determined by
nonlinear regression analysis and converted to K_i values
30 according to the equation, $K_i = IC_{50}/(1 + [L]/K_d)$, where
[L] is the ^{125}I-PYY concentration and K_d is the equilibrium
dissociation constant of ^{125}I-PYY. Data are representative
of at least two independent experiments, with each point
measured once or in duplicate. Rank orders of affinity
35 for these and other compounds are listed separately in
Table 2.

11

Figure 7B

Competitive displacement of ^{125}I-PYY on membranes from COS-7 cells transiently expressing human Y2 receptors. Membranes were incubated with ^{125}I-PYY and increasing

5   concentrations of peptide competitors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the equation, $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ^{125}I-PYY concentration and K_d is the equilibrium

10 dissociation constant of ^{125}I-PYY. Data are representative of at least two independent experiments, with each point measured once or in duplicate. Rank orders of affinity for these and other compounds are listed separately in Table 2.

15

Figure 8 Nucleotide sequence (SEQ. I.D. No. 3) and deduced amino acid sequence (SEQ. I.D. No. 4) of the rat Y2 receptor encoded by rs5a. Nucleotides are presented in the 5′ to 3′ orientation and the coding region is

20 numbered starting from the putative initiating methionine and ending in the termination codon. Deduced amino acid sequence by translation of a long open reading frame is shown using one-letter symbols.

25 **Figure 9** Nucleotide sequence (SEQ. I.D. No. 5) and deduced amino acid sequence (SEQ. I.D. No. 6) of the rat Y2 receptor encoded by rs26a. Nucleotides are presented in the 5′ to 3′ orientation and the coding region is numbered starting from the putative initiating methionine

30 and ending in the termination codon. Deduced amino acid sequence by translation of a long open reading frame is shown using one-letter symbols.

Figure 10 Alignment of rat and human Y2 receptors:

35 nucleotide sequences. Nucleotide sequences of the coding regions of the human Y2 receptor (HumY2) and the rat Y2 receptors encoded by rs5a (RatY2a) and rs26a (RatY2b) are

shown; the nucleotide sequence of rs26a (RatY2b) is identical to rs5a (RatY2a) except where shown. Rat and human Y2 nucleotide sequences exhibit ~86% identity in the coding region.

5

Figure 11 Alignment of rat and human Y2 receptors: amino acid sequences. Complete predicted amino acid sequences of the human Y2 receptor (Hum Y2) and the rat Y2 receptor encoded by rs5a (Rat Y2a) are shown; the amino acid
10 sequence of RatY2b encoded by rs26a is identical to RatY2a except where shown. Rat and human Y2 amino acid sequences are ~94% identical overall and ~98% identical in the transmembrane domains (bracketed). Single letter abbreviations for amino acids are shown.

15

Figure 12 Localization of Rat Y2 mRNA in the rat central nervous system. Schematic diagrams of half-coronal sections through the rat brain showing the distribution of neuropeptide Y Y2 receptor mRNA obtained with
20 radiolabelled oligonucleotide probes and _in situ_ hybridization histochemistry. The stars show the location of labeled neuronal populations, and are not indicative of the number of cells observed in each area.

25

Figure 13 Effects of Gpp(NH)p on radio ligand binding to Y2 receptors. Binding data were generated from competitive displacement assays in the absence (•) or presence (o) of 100 μM Gpp(NH)p. The maximum specific
30 binding detected under control conditions (in the absence of Gpp(NH)p) was used to normalize the data. A) Human Y2 receptor transiently expressed in COS-7 cells. B) Rat Y2a receptor transiently expressed in COS-7 cells.

35 **Figure 14** Inhibition of forskolin-stimulated cAMP accumulation in intact cells stably expressing the human Y2 receptor. Functional data were derived from

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radioimmunoassay of cAMP in 293 cells stimulated with 10
μM forskolin over a 5 min period. Human PYY was tested
for agonist activity over the same period. Data were fit
to a four parameter logistic equation by nonlinear
5   regression. Data generated from stably transfected 293
cells (·) and from stably transfected NIH-3T3 cells (o).
Data shown are representative of ten (·) and two (o)
independent experiments.

10   **Figure 15** Stimulation of intracellular free calcium
concentration in intact 293 cells stably expressing the
human Y2 receptor. A) Time course. Functional data were
derived from Fura-2/AM fluorescence in 293 cells
stimulated with 1 μM human PYY at the time indicated by
15   the arrow. B) Time course. Cells were stimulated with
1 μM human PYY as in A except that 1 mM EGTA was
included in the extracellular solution. C)
Concentration/response curve for PYY-dependent
mobilization of intracellular calcium in 293 cells stably
20   transfected with the human Y2 receptor. Data were fit to
a four parameter logistic equation by nonlinear
regression. Data shown are representative of at least
two independent experiments.

25   **Figure 16.** Northern analysis of various human brain
areas. Hybridization was done under conditions of high
stringency, as described in Experimental Details. The
probe was a ^{32}P-labeled DNA fragment (specific activity 3
x 10^9 cpm/μg) corresponding to the entire coding region (
30   as shown in Figure 10) of the human NPY Y2 recepotr.
The BRL RNA ladder was used as molecular weight markers.

Figure 17. Southern analysis of genomic DNA encoding the
human NPY Y2 receptor subtype. Hybridization was done
35   under conditions of high stringency, as described in
Experimental Details. The probe was a ^{32}P-labeled DNA
fragment (specific activity 2.5 x 10^9 cpm/μg)

corresponding to the TM1-TM5 region of the human NPY Y2
receptor (as shown in Figure 11). Hind III digested λ
DNA was used as molecular weight markers.

5 <u>Figure 18.</u> Photomicrographs showing some of the controls
used for NPY Y2 oligonucleotide probe specificity (A, B),
and tissue distribution of the hybridization signal in
rat brain (C, D). **A.** Darkfield photomicrograph of the
hybridization signal obtained using the radiolabeled
10 antisense probe on COS-7 cells transfected with the rat
Y2 DNA. **B.** Hybridization signal obtained following
hybridization with the radiolabeled sense probe, also on
transfected COS-7 cells. Only the antisense probes
hybridize to the transfected cells. **C.** Brightfield
15 photomicrograph of the hybridization signal observed in
the CA3 region of the rat hippocampus. Silver grains are
found over neuronal cell bodies (arrows) in the pyramidal
cell layer (sp), but not over the stratum lucidum (slu)
or stratum radiatum (sr). **D.** Hybridization signal
20 observed over neurons (arrows) in the arcuate nucleus of
the hypothalamus. The darkly stained ependymal lining of
the third ventricle can be seen to the left of the
micrograph (asterisk).

15

Detailed Description of the Invention

Throughout this application, the following standard
abbreviations are used to indicate specific nucleotide
5   bases:

           C = cytosine   A = adenine
           T = thymine   G = guanine

This invention provides isolated nucleic acid molecules
10 which encode Y2 receptors. In one embodiment, the Y2
receptor encoded is a human Y2 receptor. In another
embodiment, the Y2 receptor encoded is a rat Y2 receptor.
As used herein, the term Y2 receptor encompasses any
amino acid sequence, polypeptide or protein having
15 substantially the same pharmacology provided for the
subject human Y2 receptor as shown in Tables 2-4 and
Figures 5A-7B. As described herein our cloned receptor
has a Y2 pharmacological profile that differs from the
NPY receptor subtypes Y1 and Y3, PYY receptor, and PP
20 receptor, and is therefore designated as the Y2 receptor.

The only NPY receptor which has been cloned to date is
the Y1 receptor gene, from mouse (Eva et al., 1992), rat
(Eva et al., 1990), and human (Larhammar et al., 1992).
25 The human Y2 receptor's greatest homology with any known
receptor disclosed in the Genbank/EMBL databases is a 42%
overall amino acid identity with the human Y1 receptor.

This invention provides isolated nucleic acid molecules
30 encoding Y2 receptors. In one embodiment, the Y2
receptor is a human Y2 receptor. In another embodiment,
the Y2 receptor is a rat Y2 receptor. As used herein,
the term "isolated nucleic acid molecule" means a nucleic
acid molecule that is a molecule in a form which does not
35 occur in nature. Examples of such an isolated nucleic
acid molecule are an RNA, cDNA, or isolated genomic DNA
molecule encoding a Y2 receptor. The human Y2 receptor

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has an amino acid sequence substantially the same as the
deduced amino acid sequence shown in Figure 2 and any
human receptor having substantially the same amino acid
sequence as the amino acid sequence shown in Figure 2 is
5   by definition a human Y2 receptor. The rat Y2 receptor
has an amino acid sequence substantially the same as the
deduced amino acid sequences shown in Figure 8 or Figure
9.   One means of isolating another Y2 receptor is to
probe a genomic library with a natural or artificially
10   designed DNA probe, using methods well known in the art.
DNA probes derived from the human and the rat receptor Y2
gene are particularly useful probes for this purpose.
DNA and cDNA molecules which encode Y2 receptors may be
used to obtain genomic DNA, cDNA or RNA from human,
15   mammalian or other animal sources, or to isolate related
cDNA or genomic clones by the screening of cDNA or
genomic libraries by methods described in more detail
below. Transcriptional regulatory elements from the 5'
untranslated region of the isolated clones, and other
20   stability, processing, transcription, translation, and
tissue specificity-determining regions from the 3' and 5'
untranslated regions of the isolated genes are thereby
obtained. Examples of a nucleic acid molecule are an
RNA, cDNA, or isolated genomic DNA molecule encoding a Y2
25   receptor. Such molecules may have coding sequences
substantially the same as the coding sequences shown in
Figures 1, 8 and 9. The DNA molecule of Figure 1 encodes
the sequence of the human Y2 receptor gene. The DNA
molecules of Figures 8 and 9 encode the sequence of two
30   rat Y2 receptor genes.

This invention further provides DNA molecules encoding Y2
receptors having coding sequences substantially the same
as the coding sequences shown in Figures 1, 8 and 9.
35   These molecules are obtained by the means described
above.

17

This invention also provides an isolated nucleic acid
molecule encoding a Y2 receptor wherein the nucleic acid
molecule encodes a receptor being characterized by an
amino acid sequence in the transmembrane region, which
5   amino acid sequence has 60% homology or higher to the
amino acid sequence in the transmembrane region of the
human Y2 receptor as shown in Figure 11.

This invention also provides purified isolated proteins
10   which are Y2 receptors.   In one embodiment, the Y2
receptor protein is a human Y2 receptor protein.   In
another embodiment, the Y2 receptor protein is a rat Y2
receptor protein.   As used herein, the term "isolated
protein" means a protein molecule free of other cellular
15   components.   Examples of such proteins are isolated
proteins having substantially the same amino acid
sequence as the amino acid sequences shown in Figures 2,
8, and 9, which are a human Y2 receptor and two rat Y2
receptors, respectively.   One means for obtaining an
20   isolated Y2 receptor is to express DNA encoding the
receptor in a suitable host, such as a bacterial, yeast,
insect or mammalian cell, using methods well known in the
art, and recovering the receptor protein after it has
been expressed in such a host, again using methods well
25   known in the art. The receptor may also be isolated from
cells which express it, in particular from cells which
have been transfected with the expression vectors
described below in more detail.

30   This invention provides vectors comprising nucleic acid
molecules such as DNA, RNA, or cDNA encoding Y2
receptors.   In one embodiment, the nucleic acid encodes
a human Y2 receptor. In another embodiment, the nucleic
acid encodes a rat Y2 receptor.   Examples of vectors are
35   viruses such as bacteriophages (such as phage lambda),
animal viruses (such as Herpes virus, Murine Leukemia
virus, and Baculovirus), cosmids, plasmids (such as

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pUC18, available from Pharmacia, Piscataway, NJ), and
other recombination vectors. Nucleic acid molecules are
inserted into vector genomes by methods well known in the
art. For example, insert and vector DNA can both be
5   exposed to a restriction enzyme to create complementary
ends on both molecules which base pair with each other
and are then ligated together with a ligase.
Alternatively, linkers can be ligated to the insert DNA
which correspond to a restriction site in the vector DNA,
10   which is then digested with the restriction enzyme which
cuts at that site. Other means are also available.
Specific examples of such plasmids are: a plasmid
comprising cDNA having a coding sequence substantially
the same as the coding sequence shown in Figure 1 and
15   designated clone CG-13 (Seq. I.D. No. 1); or a plasmid
comprising genomic DNA having a coding sequence
substantially the same as the coding sequence shown in
Figure 8 and designated clone rS5a (Seq. I.D. No. 3), or
the coding sequence shown in Figure 9 and designated
20   clone rS26a (Seq. I.D. No. 5).

This invention also provides vectors comprising nucleic
acid molecules encoding Y2 receptors, adapted for
expression in a bacterial cell, a yeast cell, an insect
25   cell or a mammalian cell which additionally comprise the
regulatory elements necessary for expression of the
nucleic acid in the bacterial, yeast, insect or mammalian
cells operatively linked to the nucleic acid encoding a
Y2 receptor as to permit expression thereof. Nucleic acid
30   having coding sequences substantially the same as the
coding sequence shown in Figure 1 may be usefully
inserted into the vectors to express human Y2 receptors.
Nucleic acid having coding sequences substantially the
same as the coding sequences shown in Figures 8 and 9 may
35   be usefully inserted into vectors to express rat Y2
receptors. Regulatory elements required for expression
include promoter sequences to bind RNA polymerase and

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transcription initiation sequences for ribosome binding.
For example, a bacterial expression vector includes a
promoter such as the lac promoter and for transcription
initiation the Shine-Dalgarno sequence and the start
5   codon AUG (Maniatis, et al., Molecular Cloning, Cold
Spring Harbor Laboratory, 1982). Similarly, a eukaryotic
expression vector includes a heterologous or homologous
promoter for RNA polymerase II, a downstream
polyadenylation signal, the start codon AUG, and a
10   termination codon for detachment of the ribosome.
Furthermore, an insect expression vector, such as
recombinant baculovirus, uses the polyhedron gene
expression signals for expression of the inserted gene in
insect cells. Such vectors may be obtained commercially
15   or assembled from the sequences described by methods well
known in the art, for example the methods described above
for constructing vectors in general. Expression vectors
are useful to produce cells that express the receptor.
Certain uses for such cells are described in more detail
20   below.

This invention further provides a plasmid adapted for
expression in a bacterial cell, a yeast cell, an insect
cell, or, in particular, a mammalian cell which comprises
25   a nucleic acid molecule encoding a Y2 receptor and the
regulatory elements necessary for expression of the
nucleic acid in the bacterial, yeast, insect, or
mammalian cell operatively linked to the nucleic acid
encoding the Y2 receptor as to permit expression thereof.
30   In one embodiment, the Y2 receptor is a human Y2
receptor. In another embodiment, the Y2 receptor is a
rat Y2 receptor. Some plasmids adapted for expression in
a mammalian cell are pSVL (available from Pharmacia,
Piscataway, NJ) and pcEXV-3 (73). One specific example
35   of such a plasmid is a plasmid adapted for expression in
a mammalian cell comprising cDNA having a coding sequence
substantially the same as the coding sequence shown in

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Figure 1 and the regulatory elements necessary for expression of the DNA in the mammalian cell which is designated pcEXV-hY2, deposited on January 27, 1994 under ATCC Accession No. 75659. Other specific examples of
5   such plasmids are plasmids adapted for expression in a mammalian cell comprising genomic DNA having coding sequences substantially the same as the coding sequences shown in Figures 8 and 9 and the regulatory elements necessary for expression of the DNA in the mammalian cell
10 which are designated pcEXV-rY2a, deposited on January 25, 1995 under ATCC Accession No. 97035; and pcEXV-rY2b, deposited on January 25, 1995 under ATCC Accession No. 97036, respectively. Those skilled in the art will readily appreciate that numerous plasmids adapted for
15 expression in a mammalian cell which comprise DNA encoding Y2 receptors and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing plasmids and adapted as appropriate to contain the regulatory elements necessary
20 to express the DNA in the mammalian cell. The plasmids may be constructed by the methods described above for expression vectors and vectors in general, and by other methods well known in the art.

25 The deposits discussed supra, and the other deposits discussed herein, were made pursuant to, and in satisfaction of, the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure with the American Type
30 Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

This invention provides a cell transfected with and expressing nucleic acid encoding a Y2 receptor. In one
35 embodiment the Y2 receptor is a human Y2 receptor. In another embodiment, the Y2 receptor is a rat Y2 receptor. An example of such a cell is a mammalian cell transfected

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with a plasmid adapted for expression in a mammalian cell, which comprises nucleic acid encoding a Y2 receptor, and the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked

5   to the nucleic acid encoding a Y2 receptor as to permit expression thereof; the protein encoded thereby expressed on the cell surface. Numerous mammalian cells may be used as hosts, including, for example, the mouse fibroblast cell NIH-3T3, CHO cells, HeLa cells, LM(tk-)

10 cells, etc. Expression plasmids such as that described _supra_ may be used to transfect cells by methods well known in the art such as calcium phosphate precipitation, or DNA encoding these Y2 receptors may be otherwise introduced into cells, e.g., by microinjection, to obtain

15 mammalian cells which comprise nucleic acid, e.g., cDNA or a plasmid, encoding a Y2 receptor.   A specific example of such cells is a cell comprising the pcEXV-hY2 plasmid adapted for expression in a mammalian cell comprising cDNA encoding the Y2 receptor and the

20 regulatory elements necessary for expression of the DNA in the mammalian cell, which is designated 293-hY2-10 and deposited on January 27, 1994 under ATCC Accession No. 11837. Another specific example of such cells is a cell comprising the pcEXV-hY2 plasmid adapted for expression

25 in a mammalian cell comprising cDNA encoding the Y2 receptor and the regulatory elements necessary for expression of the DNA in the mammalian cell, which is designated N-hY2-5 and deposited on January 25, 1995 under ATCC Accession No. CRL-11825.

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This invention provides a method for determining whether a ligand can bind specifically to a Y2 receptor which comprises contacting a cell transfected with and expressing nucleic acid encoding a Y2 receptor, the

35 protein encoded thereby is expressed on the cell surface, with the ligand under conditions permitting binding of ligands known to bind to the Y2 receptor, and detecting

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the presence of any of the ligand bound to the Y2 receptor, thereby determining whether the ligand binds specifically to the Y2 receptor. In one embodiment, the Y2 receptor is a human Y2 receptor. In another
5   embodiment, the Y2 receptor is a rat Y2 receptor.

This invention further provides a method for determining whether a ligand can bind specifically to a Y2 receptor, which comprises contacting a cell transfected with and
10   expressing nucleic acid encoding the Y2 receptor with the ligand under conditions permitting binding of ligands to such receptor, and detecting the presence of any such ligand bound to the Y2 receptor, wherein the Y2 receptor is characterized by an amino acid sequence in the
15   transmembrane region, such amino acid sequence having 60% homology or higher to the amino acid sequence in the transmembrane region of the Y2 receptor shown in Figure 11. In one embodiment, the Y2 receptor is a human Y2 receptor. In another embodiment, the Y2 receptor is a
20   rat Y2 receptor.

This invention provides a method for determining whether a ligand can bind specifically to a Y2 receptor which comprises preparing a cell extract from cells transfected
25   with and expressing nucleic acid encoding a Y2 receptor, isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction from the cell extract under conditions permitting binding of ligands to such receptor, and detecting the presence of
30   any ligand bound to the Y2 receptor, thereby determining whether the compound is capable of binding specifically to a Y2 receptor. In one embodiment, the Y2 receptor is a human Y2 receptor. In another embodiment, the Y2 receptor is a rat Y2 receptor.
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This invention also provides a method for determining whether a ligand is a Y2 receptor agonist. As used

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herein, the term "agonist" means any ligand capable of
increasing Y2 receptor functional activity. This
comprises contacting a cell transfected with and
expressing nucleic acid encoding a Y2 receptor with the
5   ligand under conditions permitting the activation of a
functional Y2 receptor response from the cell, and
detecting, by means of a bioassay, such as a second
messenger assay, an increase in Y2 receptor activity,
thereby determining whether the ligand acts as a Y2
10   receptor agonist. In one embodiment, the Y2 receptor is
a human Y2 receptor. In another embodiment, the Y2
receptor is a rat Y2 receptor.

This invention further provides a method for determining
15   whether a ligand is a Y2 receptor agonist which comprises
preparing a cell extract from cells transfected with and
expressing nucleic acid encoding a Y2 receptor, isolating
a membrane fraction from the cell extract, contacting the
membrane fraction of the extract with the ligand under
20   conditions permitting the activation of a functional Y2
receptor response, and detecting, by means of a bioassay,
such as a second messenger assay, an increase in Y2
receptor activity, thereby determining whether the ligand
is a Y2 receptor agonist. In one embodiment, the Y2
25   receptor is a human Y2 receptor. In another embodiment,
the Y2 receptor is a rat Y2 receptor.

This invention also provides a method for determining
whether a ligand a Y2 receptor antagonist. As used
30   herein, the term "antagonist" means any ligand capable of
decreasing Y2 receptor functional activity. This
comprises contacting a cell transfected with and
expressing nucleic acid encoding a Y2 receptor with the
ligand in the presence of a known Y2 receptor agonist
35   such as NPY, under conditions permitting the activation
of a functional Y2 receptor response, and detecting, by
means of a bioassay, such as a second messenger assay, a

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decrease in Y2 receptor activity, thereby determining
whether the ligand is a Y2 receptor antagonist. In one
embodiment, the Y2 receptor is a human Y2 receptor. In
another embodiment, the Y2 receptor is a rat Y2 receptor.

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This invention also provides a method for determining
whether a ligand is a Y2 receptor antagonist which
comprises preparing a cell extract from cells transfected
10   with and expressing nucleic acid encoding a Y2 receptor,
isolating a membrane fraction from the cell extract,
contacting the membrane fraction of the extract with the
ligand in the presence of a known Y2 receptor agonist,
such as NPY, under conditions permitting the activation
15   of a functional Y2 receptor response, and detecting, by
means of a bioassay, such as a second messenger assay, a
decrease in Y2 receptor activity, thereby determining
whether the ligand is a Y2 receptor antagonist. In one
embodiment, the Y2 receptor is a human Y2 receptor. In
20   another embodiment, the Y2 receptor is a rat Y2 receptor.

In one embodiment, the second messenger assays referred
to comprise measurement of intracellular cAMP.   In
another embodiment, the second messenger assays comprise
25   measurement of intracellular calcium mobilization.

In one embodiment, the nucleic acid in the cells referred
to above encodes a Y2 receptor having an amino acid
sequence substantially the same as the amino acid
30   sequence shown in Figure 2. In another embodiment, the
nucleic acid in the cells referred to above encodes a Y2
receptor having an amino acid sequence substantially the
same as the amino acid sequences shown in Figure 8 or
Figure 9. In one embodiment, the cell is a mammalian
35   cell. Preferably, the mammalian cell is non-neuronal in
origin. An example of a nonneuronal mammalian cell is a
COS-7 cell. Other examples of a non-neuronal mammalian

cells that can be used for functional assays with Y2
receptors are the 293 human embryonic kidney cells, mouse
embryonic fibroblast NIH-3T3 cells, and LM(tk-) cells.

5   The preferred method for determining whether a ligand is
       capable of binding specifically to a Y2 receptor
       comprises contacting a transfected nonneuronal mammalian
       cell (i.e. a cell that does not naturally express any
       type of NPY, PP, or PYY receptor, and thus will only
10   express such a receptor if it is transfected into the
       cell) expressing a Y2 receptor on its surface, or
       contacting a membrane preparation derived from such a
       transfected cell, with the ligand under conditions which
       are known to prevail, and thus to be associated with, _in_
15   _vivo_ binding of the ligand to and/or activation of a Y2
       receptor, and detecting the presence of any of the ligand
       being tested bound to the Y2 receptor on the surface of
       the cell, or detecting activation of the Y2 receptor,
       thereby determining whether the ligand binds to,
20   activates or inhibits the activation of the Y2 receptor.
       Activation of a Y2 receptor may be detected by means of
       a second messenger assay.   Such a response system is
       obtained by transfection of nucleic acid into a suitable
       host cell containing the desired second messenger system
25   such as phospholipase C, adenylate cyclase, guanylate
       cyclase or ion channels.   A suitable host cell can be
       isolated   from   pre-existing   cell   lines,   or   can   be
       generated by inserting appropriate components of second
       messenger systems into existing cell lines.   Such a
30·   transfected cell provides a complete response system for
       investigation or assay of the activity of Y2 receptors
       with ligands as described above.   Transfection systems
       are useful as living cell cultures for competitive
       binding assays between known or candidate drugs and
35   ligands which bind to the receptor and which are labeled
       by   radioactive,   spectroscopic   or   other   reagents.
       Membrane preparations containing the receptor isolated

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from transfected cells are also useful for Y2 receptor
activity and competitive binding assays. Functional
assays of signal transduction pathways in transfection
systems determine a ligand's efficacy of activating the
5   receptor. A transfection system constitutes a "drug
discovery system" useful for the identification of
natural or synthetic compounds with potential for drug
development that can be further modified or used directly
as therapeutic compounds to activate or inhibit the
10   natural functions of the Y2 receptor. The transfection
system is also useful for determining the affinity and
efficacy of known drugs at the Y2 receptor sites.

This invention provides a pharmaceutical composition
15   comprising an effective amount of the Y2 receptor agonist
determined by the methods described above and a
pharmaceutically acceptable carrier. As used herein, the
term "pharmaceutically acceptable carrier" encompasses
any of the standard pharmaceutical carriers, such as a
20   phosphate buffered saline solution, water, and emulsions,
such as an oil/water or water/oil emulsion, and various
types of wetting agents. In one embodiment, the Y2
receptor is a human Y2 receptor. In another embodiment,
the Y2 receptor is a rat Y2 receptor. In a further
25   embodiment, the Y2 receptor agonist is not previously
known.

This invention further provides a pharmaceutical
composition comprising an effective amount of the Y2
30   receptor antagonist determined by the methods described
above and a pharmaceutically acceptable carrier. In one
embodiment the Y2 receptor is a human Y2 receptor. In
another embodiment, the Y2 receptor is a rat Y2 receptor.
In a further embodiment, the Y2 receptor antagonist is
35   not previously known.

This invention also provides a method of screening drugs

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to identify drugs which specifically bind to a Y2
receptor on the surface of a cell which comprises
contacting a cell transfected with and expressing nucleic
acid encoding the Y2 receptor with a plurality of drugs
5   under conditions permitting binding of drugs to the Y2
receptor, and determining those drugs which bind
specifically to the cell, thereby identifying drugs which
specifically bind to a Y2 receptor. In one embodiment,
the Y2 receptor is a human Y2 receptor. In another
10   embodiment, the Y2 receptor is a rat Y2 receptor.

This invention also provides a method of screening drugs
to identify drugs which specifically bind to a Y2
receptor on the surface of a cell which comprises
15   preparing a cell extract from the cells transfected with
and expressing nucleic acid encoding the Y2 receptor,
isolating a membrane fraction from the cell extract,
contacting the membrane fraction with a plurality of
drugs under conditions permitting binding of drugs to the
20   Y2 receptor, and determining those drugs which bind
specifically to the transfected cell, thereby identifying
drugs which bind specifically to a Y2 receptor. In one
embodiment, the Y2 receptor is a human Y2 receptor. In
another embodiment, the Y2 receptor is a rat Y2 receptor.
25

This invention also provides a method of screening drugs
to identify drugs which act as Y2 receptor agonists which
comprises contacting a cell transfected with and
expressing nucleic acid encoding a Y2 receptor with a
30   plurality of drugs under conditions permitting the
activation of a functional Y2 receptor response, and
determining those drugs which activate such Y2 receptor,
using a bioassay, such as a second messenger assay,
thereby identifying drugs which act as Y2 receptor
35   agonists. In one embodiment, the Y2 receptor is a human
Y2 receptor. In another embodiment the Y2 receptor is a
rat Y2 receptor. In a further embodiment, the Y2

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receptor agonist is not previously known.

This invention provides a method of screening drugs to
identify drugs which act as agonists of a Y2 receptor
5   which comprises preparing a cell extract from cells
transfected with and expressing nucleic acid encoding a
Y2 receptor, isolating a membrane fraction from the cell
extract, contacting the membrane fraction with a
plurality of drugs under conditions permitting the
10 activation of a functional Y2 receptor response, and
determining those drugs which activate such receptor,
using a bioassay, such as a second messenger assay,
thereby identifying drugs which act as Y2 receptor
agonists. In one embodiment, the Y2 receptor is a human
15 Y2 receptor. In another embodiment, the Y2 receptor is
a rat Y2 receptor. In a further embodiment, the Y2
receptor agonist is not previously known.

This invention also provides a method of screening drugs
20 to identify drugs which as Y2 receptor antagonists which
comprises contacting a cell transfected with and
expressing nucleic acid encoding a Y2 receptor with a
plurality of drugs in the presence of a known Y2 receptor
agonist such as NPY under conditions permitting the
25 activation of a functional Y2 receptor response, and
determining those drugs which inhibit the activation of
the receptor, using a bioassay, such as a second
messenger assay, thereby identifying drugs which act as
Y2 receptor antagonists. In one embodiment, the Y2
30 receptor is a human Y2 receptor. In another embodiment,
the Y2 receptor is a rat Y2 receptor. In a further
embodiment, the Y2 receptor antagonist is not previously
known.

35 This invention provides a method of screening drugs to
identify drugs which act as Y2 receptor antagonists which
comprises preparing a cell extract from cells transfected

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with and expressing nucleic acid encoding a Y2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs in the presence of a known Y2 receptor agonist,

5 such as NPY, under conditions permitting the activation of a functional Y2 receptor response, and determining those drugs which inhibit the activation of the receptor using a bioassay, such as a second messenger assay, thereby identifying drugs which act as Y2 receptor

10 antagonists. In one embodiment, the Y2 receptor is a human Y2 receptor. In another embodiment, the Y2 receptor is a rat Y2 receptor. In a further embodiment, the Y2 receptor antagonist is not previously known.

15 In one embodiment of the above described methods, the second messenger assay comprises measurement of intracellular cAMP. In another embodiment, the second messenger assay comprises measurement of intracellular calcium mobilization.

20
The nucleic acid in the cells of the methods described above may have a coding sequence substantially the same as the coding sequences shown in Figures 1, 8 and 9. Preferably, the mammalian cell is nonneuronal in origin.

25 An example of a nonneuronal mammalian cell is an COS-7 cell. Other examples of a non-neuronal mammalian cell to be used for functional assays are 293 human embryonic kidney cells, mouse embryonic fibroblast NIH-3T3 cells and LM(tk-) cells. Drug candidates are identified by

30 choosing chemical compounds which bind with high affinity to the expressed Y2 receptor protein in transfected cells, using radioligand binding methods well known in the art, examples of which are shown in the binding assays described herein. Drug candidates are also

35 screened for selectivity by identifying compounds which bind with high affinity to the Y2 receptor but do not bind with high affinity to any other NPY receptor subtype

30

or to any other known receptor site. Because selective, high affinity compounds interact primarily with the target Y2 receptor site after administration to the patient, the chances of producing a drug with unwanted
5   side effects are minimized by this approach.

This invention provides a pharmaceutical composition comprising an effective amount of a drug identified by the methods described above and a pharmaceutically
10 acceptable carrier.

As used herein, an "effective amount" is an amount of the drug effective to produce the desired result in a subject when administered in accordance with the chosen regimen.
15 Once the candidate drug has been shown to be adequately bio-available following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action for an adequate period to gain the
20 desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models, the drug may be administered to patients by that route of administration determined to make the drug bio-available, in an appropriate solid or solution
25 formulation, to gain the desired therapeutic benefit.

This invention also provides a method of treating an abnormality in a subject, wherein the abnormality is alleviated by activation of a Y2 receptor which comprises
30 administering to a subject an effective amount of the pharmaceutical composition described above, thereby treating the abnormality. In one embodiment, the Y2 receptor is a human Y2 receptor. In another embodiment, the Y2 receptor is a rat Y2 receptor.
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As used herein, the term "effective amount" means that amount of a drug which is able to produce the desired

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result in a subject when administered in accordance with
the chosen regimen. Typically, an effective amount is an
amount from about 0.01 mg per subject per day to about
500 mg per subject per day. More typically this amount
5   is an amount from about 0.1 mg per subject per day to
about 60 mg per subject per day. Most typically, this
amount is an amount from about 1 mg per subject per day
to about 20 mg per subject per day. Optimal dosages to
be administered may be determined by those skilled in the
10 art, and will vary with the particular drug in use, the
strength of the preparation, the mode of administration,
and the advancement of the disease condition. Additional
factors depending on the particular subject being treated
will result in a need to adjust dosages, including
15 subject age, weight, gender, diet, and time of
administration.

This invention provides a method of treating an
abnormality in a subject wherein the abnormality is
20 alleviated by activation of a Y2 receptor which comprises
administering to a subject an effective amount of a Y2
receptor agonist determined by the methods described
above, thereby treating the abnormality. In one
embodiment, the Y2 receptor is a human Y2 receptor. In
25 another embodiment, the Y2 receptor is a rat Y2 receptor.

This invention further provides a method of treating an
abnormality in a subject, wherein the abnormality is
alleviated by decreasing the activity of a Y2 receptor
30 which comprises administering to a subject an effective
amount of the pharmaceutical composition described above,
thereby treating the abnormality. In one embodiment, the
Y2 receptor is a human Y2 receptor. In another
embodiment, the Y2 receptor is a rat Y2 receptor.
35

This invention also provides a method of treating an
abnormality in a subject, wherein the abnormality is

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alleviated by decreasing the activity of a Y2 receptor
which comprises administering to the subject an effective
amount of a Y2 receptor antagonist determined by the
methods described above, thereby treating the
5 abnormality. In one embodiment, the Y2 receptor is a
human Y2 receptor. In another embodiment, the Y2
receptor is a rat Y2 receptor.

This invention provides a nucleic acid probe comprising
10 a nucleic acid molecule of at least 15 nucleotides
capable of specifically hybridizing with a unique
sequence included within the sequence of a nucleic acid
molecule encoding a Y2 receptor, for example with a
coding sequence included within the sequences shown in
15 Figures 1, 8 and 9. As used herein, the phrase
"specifically hybridizing" means the ability of a nucleic
acid molecule to recognize a nucleic acid sequence
complementary to its own and to form double-helical
segments through hydrogen bonding between complementary
20 base pairs. As used herein, a "unique sequence" is a
sequence specific to only the nucleic acid molecules
encoding the Y2 receptor. In one embodiment the Y2
receptor is a human Y2 receptor. In another embodiment,
the Y2 receptor is a rat Y2 receptor. Nucleic acid probe
25 technology is well known to those skilled in the art who
will readily appreciate that such probes may vary greatly
in length and may be labeled with a detectable label,
such as a radioisotope or fluorescent dye, to facilitate
detection of the probe. Detection of nucleic acid
30 encoding Y2 receptors is useful as a diagnostic test for
any disease process in which levels of expression of the
corresponding Y2 receptor is altered. DNA probe
molecules are produced by insertion of a DNA molecule
which encodes Y2 receptor or fragments thereof into
35 suitable vectors, such as plasmids or bacteriophages,
followed by insertion into suitable bacterial host cells
and replication and harvesting of the DNA probes, all

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using methods well known in the art. For example, the
DNA may be extracted from a cell lysate using phenol and
ethanol, digested with restriction enzymes corresponding
to the insertion sites of the DNA into the vector
5   (discussed above), electrophoresed, and cut out of the
resulting gel. Examples of such DNA molecules are shown
in Figures 1, 8 and 9. The probes are useful for 'in
situ' hybridization or in order to locate tissues which
express this gene family, or for other hybridization
10 assays for the presence of these genes or their mRNA in
various biological tissues. In addition, synthesized
oligonucleotides (produced by a DNA synthesizer)
complementary to the sequence of a DNA molecule which
encodes a Y2 receptor are useful as probes for these
15 genes, for their associated mRNA, or for the isolation of
related genes by homology screening of genomic or cDNA
libraries, or by the use of amplification techniques such
as the Polymerase Chain Reaction. Synthesized
oligonucleotides as described may also be used to
20 determine the cellular localization of the mRNA produced
by the Y2 gene by in situ hybridization.

This invention also provides a method of detecting
expression of a Y2 receptor by detecting the presence of
25 mRNA coding for a Y2 receptor which comprises obtaining
total mRNA from the cell using methods well known in the
art and contacting the mRNA so obtained with a nucleic
acid probe comprising a nucleic acid molecule of at least
15 nucleotides capable of specifically hybridizing with
30 a sequence included within the sequence of a nucleic acid
molecule encoding the Y2 receptor under hybridizing
conditions, and detecting the presence of mRNA hybridized
to the probe, thereby detecting the expression of the Y2
receptor by the cell. In one embodiment, the Y2 receptor
35 is a human Y2 receptor. In another embodiment, the Y2
receptor is a rat Y2 receptor. Hybridization of probes
to target nucleic acid molecules such as mRNA molecules

34

employs techniques well known in the art. In one
possible means of performing this method, nucleic acids
are extracted by precipitation from lysed cells and the
mRNA is isolated from the extract using a column which
5 binds the poly-A tails of the mRNA molecules. The mRNA
is then exposed to radioactively labelled probe on a
nitrocellulose membrane, and the probe hybridizes to and
thereby labels complementary mRNA sequences. Binding may
be detected by autoradiography or scintillation counting.
10 However, other methods for performing these steps are
well known to those skilled in the art, and the
discussion above is merely an example.

This invention provides an antisense oligonucleotide
15 having a sequence capable of specifically hybridizing to
an mRNA molecule which encodes a Y2 receptor so as to
prevent translation of the mRNA molecule. The antisense
oligonucleotide may have a sequence capable of
specifically hybridizing with the cDNA molecule whose
20 sequence is shown in Figure 1, or with the genomic DNA
molecule whose sequences are shown in Figures 8 and 9.
A particular example of an antisense oligonucleotide is
an antisense oligonucleotide comprising chemical
analogues of nucleotides.
25   ‐

This invention also provides a pharmaceutical composition
comprising an amount of the oligonucleotide described
above effective to decrease activity of a Y2 receptor by
passing through a cell membrane and specifically
30 hybridizing with mRNA encoding a Y2 receptor in the cell
so as to prevent its translation and a pharmaceutically
acceptable carrier capable of passing through a cell
membrane. The oligonucleotide may be coupled to a
substance which inactivates mRNA, such as a ribozyme.
35 The pharmaceutically acceptable carrier capable of
passing through cell membranes may also comprise a
structure which binds to a receptor specific for a

selected cell type and is thereby taken up by cells of
the selected cell type. The structure may be part of a
protein known to bind a cell-type specific receptor, for
example an insulin molecule, which would target

5   pancreatic cells. In one embodiment, the Y2 receptor is
a human Y2 receptor. In another embodiment, the Y2
receptor is a rat Y2 receptor. DNA molecules having
coding sequences substantially the same as the coding
sequences shown in Figures 1, 8 and 9 may be used as the

10   oligonucleotides of the pharmaceutical composition.

This invention also provides a method of treating an
abnormality in a subject wherein the abnormality is
alleviated by decreasing the activity of a Y2 receptor

15   which comprises administering to the subject an effective
amount of the pharmaceutical composition described above,
thereby treating the abnormality. In one embodiment, the
Y2 receptor is a human Y2 receptor. In another
embodiment, the Y2 receptor is a rat Y2 receptor.

20   Several examples of such abnormalities are hypertension,
gastrointestinal disorders, epilepsy, sleep disorders,
and cognitive disorders, (58-80).

Antisense oligonucleotide drugs inhibit translation of

25   mRNA encoding these receptors. Synthetic
oligonucleotides, or other antisense chemical structures
are designed to bind to mRNA encoding the Y2 receptor and
inhibit translation of mRNA and are useful as drugs to
inhibit expression of Y2 receptor genes in patients.

30   This invention provides a means to therapeutically alter
levels of expression of Y2 receptors by the use of a
synthetic antisense oligonucleotide drug (SAOD) which
inhibits translation of mRNA encoding these receptors.
Synthetic oligonucleotides, or other antisense chemical

35   structures designed to recognize and selectively bind to
mRNA, are constructed to be complementary to portions of
the nucleotide sequences shown in Figures 1, 8, and 9 of

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DNA, RNA or of chemically modified, artificial nucleic acids. The SAOD is designed to be stable in the blood stream for administration to patients by injection, or in laboratory cell culture conditions, for administration to
5   cells removed from the patient. The SAOD is designed to be capable of passing through cell membranes in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOD which render it capable of passing through cell membranes (e.g. by designing
10 small, hydrophobic SAOD chemical structures) or by virtue of specific transport systems in the cell which recognize and transport the SAOD into the cell. In addition, the SAOD can be designed for administration only to certain selected cell populations by targeting the SAOD to be
15 recognized by specific cellular uptake mechanisms which binds and takes up the SAOD only within certain selected cell populations. For example, the SAOD may be designed to bind to a receptor found only in a certain cell type, as discussed above. The SAOD is also designed to
20 recognize and selectively bind to the target mRNA sequence, which may correspond to a sequence contained within the sequences shown in Figures 1, 8, and 9 by virtue of complementary base pairing to the mRNA. Finally, the SAOD is designed to inactivate the target
25 mRNA sequence by any of three mechanisms: 1) by binding to the target mRNA and thus inducing degradation of the mRNA by intrinsic cellular mechanisms such as RNAse I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-
30 regulating factors or of ribosomes, or 3) by inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense oligonucleotide drugs have been shown to be capable of
35 the properties described above when directed against mRNA targets (74,75). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for

37

inactivating target mRNA (76). An SAOD serves as an effective therapeutic agent if it is designed to be administered to a patient by injection, or if the patient's target cells are removed, treated with the SAOD
5   in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce receptor expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of Y2 receptors.
10

This invention provides an antibody directed to a Y2 receptor, for example, a monoclonal antibody directed to an epitope of a Y2 receptor present on the surface of a cell and having an amino acid sequence substantially the
15   same as an amino acid sequence for a cell surface epitope of the Y2 receptor included in the amino acid sequences shown in Figures 2, 8 and 9 (Seq. I.D. Nos. 2, 4, and 6, respectively). In one embodiment, the Y2 receptor is a human Y2 receptor. In another embodiment, the Y2
20   receptor is a rat Y2 receptor. Amino acid sequences may be analyzed by methods well known in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to
25   form the part of the protein that is inserted into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figures 2, 8,
30   and 9 will probably bind to a surface epitope of a Y2 receptor, as described. Antibodies directed to Y2 receptors may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using
35   hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired

38·

antibody. Cells such as COS-7 cells, LM(tk-) cells, NIH-
3T3 cells or 293 human embryonic cells comprising DNA
encoding the Y2 receptor and thereby expressing the Y2
receptor may be used as immunogens to raise such an
5   antibody.   Alternatively, synthetic peptides may be
prepared using commercially available machines and the
amino acid sequences shown in Figures 2, 8, and 9 (Seq.
I.D. Nos. 2, 4, and 6, respectively). As a still further
alternative, DNA, such as a cDNA or a fragment thereof,
10 may be cloned and expressed and the resulting polypeptide
recovered and used as an immunogen. These antibodies are
useful to detect the presence of Y2 receptors encoded by
the isolated DNA, or to inhibit the function of the
receptors in living animals, in humans, or in biological
15 tissues or fluids isolated from animals or humans.

This invention provides a pharmaceutical composition
which comprises an amount of an antibody directed to a Y2
receptor effective to block binding of ligands to the Y2
20 receptor, and a pharmaceutically acceptable carrier. A
monoclonal antibody directed to an epitope of a Y2
receptor present on the surface of a cell and having an
amino acid sequence substantially the same as an amino
acid sequence for a cell surface epitope of the Y2
25 receptor included in the amino acid sequences shown in
Figures 2, 8 and 9 are useful for this purpose.

This invention also provides a method of treating an
abnormality in a subject, wherein the abnormality is
30· alleviated by decreasing the activity of a Y2 receptor
which comprises administering to the subject an amount of
the pharmaceutical composition described above effective
to block binding of ligands to the Y2 receptor, thereby
treating the abnormality. In a one embodiment, the Y2
35 receptor is a human Y2 receptor. In another embodiment,
the Y2 receptor is a rat Y2 receptor. Binding of the
antibody to the receptor prevents the receptor from

39

functioning, thereby neutralizing the effects of activity of the receptor. The monoclonal antibodies described above are both useful for this purpose. Some examples of such abnormalities are hypertension, gastrointestinal

5 disorders, epilepsy, sleep disorders, and cognitive disorders (58-72).

This invention provides a method of detecting the presence of a Y2 receptor on the surface of a cell which

10 comprises contacting the cell with an antibody directed to the Y2 receptor, under conditions permitting binding of the antibody to the receptor, and detecting the presence of the antibody bound to the cell, thereby detecting the presence of a Y2 receptor on the surface of

15 the cell. Such a method is useful for determining whether a given cell is defective in expression of Y2 receptors on the surface of the cell. Bound antibodies are detected by methods well known in the art, for example by binding fluorescent markers to the antibodies

20 and examining the cell sample under a fluorescence microscope to detect fluorescence on a cell indicative of antibody binding. The monoclonal antibodies described above are useful for this purpose.

25 This invention provides a transgenic nonhuman mammal expressing nucleic acid encoding a Y2 receptor. In one embodiment, the Y2 receptor is a human Y2 receptor. In another embodiment, the Y2 receptor is a rat Y2 receptor. This invention also provides a transgenic nonhuman mammal

30 comprising a homologous recombination knockout of the native Y2 receptor. This invention also provides a transgenic nonhuman mammal whose genome comprises antisense nucleic acid complementary to nucleic acid encoding a Y2 receptor so placed as to be transcribed

35 into antisense mRNA which is complementary to mRNA encoding a Y2 receptor and which hybridizes to mRNA encoding a Y2 receptor thereby reducing its translation.

40

The nucleic acid may additionally comprise an inducible promoter or additionally comprise tissue specific regulatory elements, so that expression can be induced, or restricted to specific cell types. Examples of
5 nucleic acid are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequences shown in Figures 1, 8, and 9. An example of a transgenic animal is a transgenic mouse. Examples of tissue specificity-determining regions are the metallothionein
10 promotor (77) and the L7 promotor (78).

Animal model systems which elucidate the physiological and behavioral roles of Y2 receptors are produced by creating transgenic animals in which the activity of a Y2
15 receptor is either increased or decreased, or the amino acid sequence of the expressed Y2 receptor protein is altered, by a variety of techniques. Examples of these techniques include: 1) Insertion of normal or mutant versions of nucleic acid encoding a Y2 receptor or
20 homologous animal versions of these genes, by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal (79).   2) Homologous recombination (80, 81) of
25 mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these Y2 receptors. The technique of homologous recombination is well known in the art. It replaces the native gene with
30 the inserted gene and so is useful for producing an animal that cannot express native receptor but does express, for example, an inserted mutant receptor, which has replaced the native receptor in the animal's genome by recombination, resulting in underexpression of the
35 receptor. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added receptors,

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resulting in overexpression of the receptor. One means
available for producing a transgenic animal, with a mouse
as an example, is as follows: Female mice are mated,
and the resulting fertilized eggs are dissected out of
5   their oviducts. The eggs are stored in an appropriate
medium such as M2 medium (79). DNA or cDNA encoding a Y2
receptor is purified from a vector (such as plasmid
pcEXV-hY2, pcEXV-rY2a or pcEXV-rY2b described above) by
methods well known in the art. Inducible promoters may be
10   fused with the coding region of the nucleic acid to
provide an experimental means to regulate expression of
the trans-gene. Alternatively, or in addition, tissue
specific regulatory elements may be fused with the coding
region to permit tissue-specific expression of the trans-
15   gene. The nucleic acid, in an appropriately buffered
solution, is put into a microinjection needle (which may
be made from capillary tubing using a pipet puller) and
the egg to be injected is put in a depression slide. The
needle is inserted into the pronucleus of the egg, and
20   the nucleic acid solution is injected. The injected egg
is then transferred into the oviduct of a pseudopregnant
mouse (a mouse stimulated by the appropriate hormones to
maintain pregnancy but which is not actually pregnant),
where it proceeds to the uterus, implants, and develops
25   to term. As noted above, microinjection is not the only
method for inserting nucleic acid into the egg cell, and
is used here only for exemplary purposes.

Since the normal action of receptor-specific drugs is to
30   activate or to inhibit the receptor, the transgenic
animal model systems described above are useful for
testing the biological activity of drugs directed against
these Y2 receptors even before such drugs become
available. These animal model systems are useful for
35   predicting   or   evaluating   possible   therapeutic
applications of drugs which activate or inhibit these Y2
receptors by inducing or inhibiting expression of the

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native or trans-gene and thus increasing or decreasing
activity of normal or mutant Y2 receptors in the living
animal. Thus, a model system is produced in which the
biological activity of drugs directed against these Y2

5   receptors are evaluated before such drugs become
available. The transgenic animals which over or under
produce the Y2 receptor indicate by their physiological
state whether over or under production of the Y2 receptor
is therapeutically useful. It is therefore useful to

10 evaluate drug action based on the transgenic model
system. One use is based on the fact that it is well
known in the art that a drug such as an antidepressant
acts by blocking neurotransmitter uptake, and thereby
increases the amount of neurotransmitter in the synaptic

15 cleft.   The physiological result of this action is to
stimulate the production of less receptor by the affected
cells, leading eventually to decreased activity.
Therefore, an animal which has decreased receptor
activity is useful as a test system to investigate

20 whether the actions of such drugs which result in
decreased activity are in fact therapeutic. Another use
is that if increased activity is found to lead to
abnormalities, then a drug which down-regulates or acts
as an antagonist to a Y2 receptor is indicated as worth

25 developing, and if a promising therapeutic application is
uncovered by these animal model systems, activation or
inhibition of the Y2 receptor is achieved therapeutically
either by producing agonist or antagonist drugs directed
against these Y2 receptors or by any method which

30 increases or decreases the activity of these Y2 receptors
in humans or other mammals.

This invention provides a method of determining the
physiological effects of expressing varying levels of Y2

35 receptors which comprises producing a transgenic nonhuman
animal whose levels of Y2 receptor expression are varied
by use of an inducible promoter which regulates Y2

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receptor expression. This invention also provides a method of determining the physiological effects of expressing varying levels of Y2 receptors which comprises producing a panel of transgenic nonhuman animals each
5   expressing a different amount of Y2 receptor. In one embodiment, the Y2 receptor is a human Y2 receptor. In another embodiment, the Y2 receptor is a rat Y2 receptor. Such animals may be produced by introducing different amounts of nucleic acid encoding a Y2 receptor into the
10   oocytes from which the transgenic animals are developed.

This invention also provides a method for identifying a Y2 receptor antagonist capable of alleviating an abnormality is a subject, wherein the abnormality is
15   alleviated by decreasing the acitivity of a Y2 receptor which comprises administering the antagonist to a transgenic nonhuman mammal described above and determining whether the antagonist alleviates the physical and behavioral abnormalities displayed by the
20   transgenic nonhuman mammal as a result of the activity of a Y2 receptor, thereby identifying a Y2 antagonist. In one embodiment, the Y2 receptor is a human Y2 receptor, In another embodiment, the Y2 receptor is a rat Y2 receptor. This invention further provides an antagonist
25   identified by the method described above. Examples of nucleic acid molecules are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequences shown in Figures 1, 8, and 9.

30   This invention provides a pharmaceutical composition comprising an amount of the antagonist described supra effective to alleviate an abnormality wherein the abnormality is alleviated by decreasing the activity of a Y2 receptor and a pharmaceutically acceptable carrier.
35

This invention further provides a method for treating an abnormality in a subject wherein the abnormality is

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alleviated by decreasing the activity of a Y2 receptor
which comprises administering to the subject an effective
amount of the pharmaceutical composition described above,
thereby treating the abnormality.

5

This invention provides a method for identifying a Y2
receptor agonist capable of alleviating an abnormality
wherein the abnormality is alleviated by activation of a
Y2 receptor which comprises administering the agonist to

10   the transgenic nonhuman mammal described above and
determining whether the agonist alleviates the physical
and behavioral abnormalities displayed by the transgenic
nonhuman mammal, thereby identifying a Y2 receptor
agonist. In one embodiment, the Y2 receptor is a human

15   Y2 receptor. In another embodiment, the Y2 receptor is
a rat Y2 receptor. This invention further provides an
agonist identified by the method described above.

This invention also provides a pharmaceutical composition

20   comprising an effective amount of a Y2 receptor agonist
identified by the method described above and a
pharmaceutically acceptable carrier.

This invention further provides a method for treating an

25   abnormality in a subject wherein the abnormality is
alleviated by activation of a Y2 receptor which comprises
administering to the subject an effective amount of the
pharmaceutical composition described above, thereby
treating the abnormality.

30

This invention provides a method for diagnosing a
predisposition to a disorder associated with the
expression of a specific Y2 receptor allele which
comprises: a) obtaining nucleic acid of subjects

35   suffering from the disorder; b) performing a restriction
digest of the nucleic acid with a panel of restriction
enzymes; c) electrophoretically separating the resulting

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nucleic acid fragments on a sizing gel; d) contacting the
resulting gel with a nucleic acid probe capable of
specifically hybridizing to nucleic acid encoding a Y2
receptor and labelled with a detectable marker; e)
5   detecting labelled bands which have hybridized to the
nucleic acid encoding a Y2 receptor labelled with a
detectable marker to create a unique band pattern
specific to the nucleic acid of subjects suffering from
the disorder; f) preparing nucleic acid obtained for
10   diagnosis by steps a-e; and g) comparing the unique band
pattern specific to the nucleic acid of subjects
suffering from the disorder from step e and the nucleic
acid obtained for diagnosis from step f to determine
whether the patterns are the same or different and
15   thereby to diagnose predisposition to the disorder if the
patterns are the same. This method may also be used to
diagnose a disorder associated with the expression of a
specific Y2 receptor allele. In one embodiment, the Y2
receptor is a human Y2 receptor. In another embodiment,
20   the Y2 receptor is a rat Y2 receptor.

This invention provides a method of preparing the
isolated, purified Y2 receptor which comprises a)
constructing a vector adapted for expression in a cell
25   which comprises the regulatory elements necessary for the
expression of nucleic acid in the cell operatively linked
to the nucleic acid encoding a Y2 receptor as to permit
expression thereof., wherein the cell is selected form the
group consisting of bacterial cells, yeast cells, insect
30   cells and mammalian .cells; b) inserting the vector of
step (a) in a suitable host cell; c) incubating the cells
of step (b) under conditions allowing the expression of
a Y2 receptor; d) recovering the receptor so produced;
and e) purifying the receptor so recovered. An example
35   of an isolated Y2 receptor is an isolated protein having
substantially the same amino acid sequence as the amino
acid sequences shown in Figures 2, 8 and 9.   For

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example, cells can be induced to express receptors by
exposure to substances such as hormones. The cells can
then be homogenized and the receptor isolated from the
homogenate using an affinity column comprising, for
5   example, PYY or NPY or another substance which is known
to bind to the receptor. The resulting fractions can
then be purified by contacting them with an ion exchange
column, and determining which fraction contains receptor
activity or binds anti-receptor antibodies.
10

The above described method for preparing a Y2 receptor
uses recombinant DNA technology methods well known in the
art. For example, isolated nucleic acid encoding Y2
receptor is inserted in a suitable vector, such as an
15   expression vector. A suitable host cell, such as a
bacterial cell, or a eukaryotic cell such as a yeast
cell, is transfected with the vector. Y2 receptor is
isolated from the culture medium by affinity purification
or by chromatography or by other methods well known in
20   the art.

This invention identifies for the first time a new
receptor protein, its amino acid sequence, its human gene
and its rat homologue. Furthermore, this invention
25   describes a previously unrecognized group of receptors
within the definition of a Y2 receptor. The information
and experimental tools provided by this discovery are
useful to generate new therapeutic agents, and new
therapeutic or diagnostic assays for this new receptor
30   protein, its associated mRNA molecule or its associated
genomic DNA. The information and experimental tools
provided by this discovery will be useful to generate new
therapeutic agents, and new therapeutic or diagnostic
assays for this new receptor protein, its associated mRNA
35   molecule, or its associated genomic DNA.

Specifically, this invention relates to the first

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isolation of a human genomic clone encoding a Y2 receptor. A new human gene for the receptor identified herein as Y2 has been identified and characterized. In addition, the human Y2 receptor has been expressed in 293

5   human embryonic kidney cells. The pharmacological binding properties of the protein encoded have been determined, and these binding properties classify this protein as a novel human NPY/PYY receptor which we designate as a human Y2 receptor. Mammalian cell lines

10 expressing this human Y2 receptor at the cell surface have been constructed, thus establishing the first well-defined, cultured cell lines with which to study this Y2 receptor.

15 This invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which

20 follow thereafter.

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EXPERIMENTAL DETAILS

cDNA Cloning

Total RNA was prepared by a modification of the guanidine
5   thiocyanate method (13), from 6 grams of human
hippocampus. Poly A$^+$RNA was purified with a FastTrack kit
(Invitrogen Corp., San Diego, CA). Double stranded (ds)
cDNA was synthesized from 4 μg of poly A$^+$ RNA according to
Gubler and Hoffman (14), except that ligase was omitted
10   in the second strand cDNA synthesis. The resulting DS
cDNA was ligated to BstxI/EcoRI adaptors (Invitrogen
Corp.), the excess of adaptors was removed by
chromatography on Sephacryl 500 HR (Pharmacia-LKB) and
the ds-cDNA size selected by chromatography on Sephacryl
15   1000 (Pharmacia-LKB). High molecular weight fractions
were ligated in pcEXV.BS (An Okayama and Berg expression
vector) cut by BstxI as described by Aruffo and Seed
(15). The ligated DNA was electroporated in E. coli MC
1061 (Gene Pulser, Biorad). A total of 2.2 x 10^6
20   independent clones with an insert mean size of 3 kb could
be generated. The library was plated on Petri dishes
(Ampicillin selection) in pools of 0.4 to 1.2 x 10^4
independent clones. After 18 hours amplification, the
bacteria from each pool were scraped, resuspended in 4 mL
25   of LB media and 1.5 mL processed for plasmid purification
by the alkali method (16). 1 mL aliquots of each
bacterial pool were stored at -85°C in 20% glycerol.

Isolation of a cDNA clone encoding a human hippocampal Y2
30   receptor.

DNA from pools of ≈ 5000 independent clones was
transfected into COS-7 cells by a modification of the
DEAE-dextran procedure (17). COS-7 cells were grown in
Dulbecco's Modified Eagle Medium (DMEM) supplemented with
35   10% fetal calf serum, 100 U/mL of penicillin, 100 μg/mL
of streptomycin, 2mM L-glutamine (DMEM-C) at 37°c in 5%
CO_2. The cells were seeded one day before transfection at

a density of 30,000 cells/cm^2 in 6 well plates (Becton Dickinson, Lincoln Park, NJ). On the next day, cells were washed twice with Phosphate Buffer Saline (PBS), 400 μl of transfection cocktail was added containing 1/10 of
5   the DNA from each pool and DEAE-dextran (500 μg/mL) in PBS. After a 30 min. incubation at 37°C, 1.6 mL of chloroquine (80 μM in DMEM-C) was added and the cells incubated a further 2.5 hours at 37°C. The media was aspirated from each well and 1 mL of 10% DMSO in DMEM-C
10   added. After 2.5 min. incubation at room temperature, the media was aspirated, each well washed once with 1 mL PBS and the cells incubated 24 hours in DMEM-C. The cells were then trypsinized and seeded on Lab-Tek chamber slides (1 chamber, Permanox slide from Nunc Inc.,
15   Naperville, IL), incubated in 2 ml DMEM-C for another 24 hours and the binding assay was performed on the slides.

After two washes with PBS, positive pools were identified by incubating the cells with 1 nM (3x10^6 cpm per slide) of
20   porcine [^{125}I]-PYY (New England Nuclear; specific activity=2200Ci/mmol) in 20 mM Hepes-NaOH pH 7.4, CaCl$_2$ 1.26 mM, MgSO$_4$ .81 mM, KH$_2$PO$_4$ .44 mM, KCl 5.4, NaCl 10mM, .1% bovine serum albumin, .1% bacitracin for 1 hour at room temperature. After six washes (five seconds each)
25   in binding buffer without ligand, the monolayers were fixed in 2.5% glutaraldehyde in PBS for five minutes, washed twice two minutes in PBS, dehydrated in ethanol baths for two minutes each (70, 80, 95, 100%) and air dried.
30
The slides were then dipped in 100% photoemulsion (Kodak type NTB2) at 42°C and exposed in the dark for 48 hours at 4°C in light proof boxes containing drierite. Slides were developed for three minutes in Kodak D19 developer
35   (32 g/l of water), rinsed in water, fixed in Kodak fixer for 5 minutes, rinsed in water, air dried and mounted with Aqua-Mount (Lerner Laboratories, Pittsburgh, PA).

50

Slides were screened at 25x total magnification.

A single clone, CG-13, was isolated by sib selection as
described (18). DS-DNA was sequenced with a Sequenase
5   kit (US Biochemical, Cleveland, OH) according to the
manufacturer. Nucleotide and peptide sequences analysis
were performed with GCG programs (Genetics Computer
group, Madison, WI).

10 **Northern Blot**
A multiple tissue Northern blot (MTN blot, Contech, Palo
Alto, CA) carrying mRNA purified from various human brain
areas was hybridized at high stringency according to the
manufacturer's specifications. The probe was a 1.15 kb
15 DNA fragment corresponding to the entire coding region of
the human Y2 receptor as shown in Figure 10.

Southern Blot:
A Southern blot (Geno-Blot, Clontech, Palo Alto, CA)
20 containing human genomic DNA cut with five different
enzymes (8 μg DNA per lane) was hybridized at high
stringency   according   to   the   manufacturer's
specifications.   The .probe was a DNA fragment
corresponding to the TM1-TM5 coding region of the human
25 Y2 receptor, as shown in Figure 11.

**Cloning and Expression of Two Isoforms of the Rat NPY/PYY
(Y2) Receptor**
To obtain the rat homologue of the human NPY/PYY (Y2)
30 receptor, we designed and synthesized oligonucleotide
probes   derived   from   the   nucleotide   sequences
corresponding approximately to the transmembrane (TM)
regions of the amino acid sequence of the human Y2
receptor (TM 1 - 7) as shown in Figure 11. The
35 overlapping oligomers used were as follows:

(**TM1:**   nts.   #190-257,   (+)   strand/5'-

CAAGTTGTTCTCATATTGGCCTACTGCTCCATCATCTTGCTTGGGGTAAT-3'
(Seq. I.D. No. 7) and (-) strand/5'-
ATCACCACATGGATCACCAAGGAGTTGCCAATTACCCCAAGCAAGATGAT-3'
(Seq. I.D. No. 8)

5

TM2: nts. #301-370, (+) strand/5'-
TTTTTCATTGCCAATCTGGCTGTGGCAGATCTTTTGGTGAACACT-3' (Seq.
I.D. No. 9) and (-) strand/5'-
AGGTAAGAGTGAACGGTAGACACAGAGTGTTCACCAAAAGATCTG-3' (Seq.
10 I.D. No. 10).

TM3: nts. #411-480, (+) strand/5'-
CCACCTGGTGCCCTATGCCCAGGGCCTGGCAGTACAAGTATCCAC-3' (Seq.
I.D. No. 11) and (-) strand/5'-
15 CAGGGCAATTACTGTCAAGGTGATTGTGGATACTTGTACTGCCAG-3' (Seq. I.
D. No. 12).

TM4: nts. #531-600, (+) strand/5'-
AATCAGCTTCCTGATTATTGGCTTGGCCTGGGGCATCAGTGCCCT-3' (Seq.
20 I.D. No. 13) and (-) strand/5'-
GAAGATGGCCAGGGGACTTGCCAGCAGGGCACTGATGCCCCAGGC-3' (Seq.
I.D. No. 14)

TM5: nts. #691-760, (+) strand/5'-
25 ACTGTCTATAGTCTTTCTTCCTTGTTGATCTTGTATGTTTTGCCT-3' (Seq.
I.D. No. 15) and (-) strand/5'-
TGTAGGAAAATGATATAATGCCCAGAGGCAAAACATACAAGATCA-3' (Seq.
I.D. NO. 16)

30 **TM6:** nts. #850-919, (+) strand/5'-
CTGGTGTGTGTGGTGGTGGTGTTTGCGGTCAGCTGGCTGCCTCTC-3' (Seq.
I.D. No. 17) and (-) strand/5'-
TGTCAACGGCAAGCTGGAAGGCATGGAGAGGCAGCCAGCTGACCG-3' (Seq.
I.D. No. 18)

35

TM7: NTS. #955-1028, (+) strand/5'-
CTCATCTTCACAGTGTTCCACATCATCGCCATGTGCTCCACTTTTGC-3' (Seq.

52

I.D. No. 19) and (-) strand/5'-

TTCATCCAGCCATAGAGAAGGGGATTGGCAAAAGTGGAGCACATGGC-3' (Seq.

I.D. No. 20).

5   The probes were labeled with [^{32}P]-ATP and [^{32}p]-CTP by

synthesis with the large fragment of DNA polymerase.

53

Hybridization was performed at 40°C in a solution
containing 25% formamide, 10% dextran sulfate, 5X SSC (1X
SSC is 0.15 M sodium chloride, 0.015 M sodium citrate),
1X Denhardt's (0.02% polyvinylpyrrolidone, 0.02% Ficoll,
5 and 0.02% bovine serum albumin), and 100 μg/ml of
sonicated salmon sperm DNA. The filters were washed at
40°C in 0.1X SSC containing 0.1% sodium dodecyl sulfate
(SDS) and exposed at -70°C to Kodak XAR film in the
presence of one intensifying screen.   Lambda phage
10 hybridizing to the probes were plaque purified by
successive plating and rescreening. A genomic clone
hybridizing with six out of seven TM probes, designated
rs5a, was isolated using this method. A 4.0 kb EcoRI
fragment of rs5a was subcloned into the eukaryotic
15 expression vector EXJ.RH modified from pcEXV-3 (73) for
sequence analysis and expression studies. The nucleotide
sequence of the fragment in EXJ.RH was analyzed on both
strands by the Sanger dideoxy nucleotide chain-
termination method (82) using Sequenase (U.S. Biochemical
20 Corp., Cleveland, Ohio).

A second genomic clone, termed rs26a, was also isolated
using the hybridization conditions described above and
exhibited the same hybridization profile with TM probes.
25 In contrast with rs5a, however, rs26a contained an
internal EcoRI restriction enzyme site not present in
the other clone.   To further investigate potential
differences between the two clones, a 3.9 kb SalI/KpnI
fragment of rs26a was subcloned into the expression
30 · vector EXJ.HR for sequence analysis and expression
studies. The nucleotide sequence of the fragment was
analyzed on both strands by the Sanger dideoxy nucleotide
chain-termination method as described above.

35 **Cell Culture**
COS-7 cells were grown on 150 mm plates in Dulbecco's
Modified Eagle Medium (DMEM) with supplements (10% bovine

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calf serum, 4 mM glutamine, 100 units/ml penicillin/100 μg/ml streptomycin) at 37 °C, 5% CO_2. Stock plates of COS-7 cells were trypsinized and split 1:6 every 3-4 days.

5   Human embryonic kidney cells 293 cells were grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 μg/ml streptomycin) at 37 °C, 5% CO_2. Stock plates of 293 cells were trypsinized and split
10 1:6 every 3-4 days.

Mouse embryonic fibroblast NIH-3T3 cells were grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine,
15 100 units/ml penicillin/100 μg/ml streptomycin) at 37 °C, 5% CO_2. Stock plates of NIH-3T3 cells were trypsinized and split 1:15 every 3-4 days.

SK-N-Be(2) human neuroblastoma cells were grown similarly
20 in 225 cm^2 flasks using 50% Eagle's Modified Essential Media, 50% Ham's Nutrient Mixture F-12, 15% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin/80 units/ml streptomycin, and 1% non-essential amino acids. Stock flasks of SK-N-Be(2) cells were trypsinized and
25 split 1:10 every 7 days.

DNA Transfection for Pharmacological Characterization
All cloned receptor subtypes studied (human Y1, human Y2, human Y4, rat Y2a and rat Y2b) were transiently
30 transfected into COS-7 cells by the DEAE-dextran method, using 1 μg of DNA/10^6 cells (17). The cDNA corresponding to the cloned Y4 receptor was disclosed in U.S. patent application 08/176,412 filed on December 28, 1993, currently pending.
35

Membrane Preparation
Membranes were harvested from COS-7 cells 48 hours after

transfection and from SK-N-Be(2) seven days after splitting. Adherent cells were washed twice in ice-cold phosphate buffered saline (138 mM NaCl, 8.1 mM Na_2HPO_4, 2.5 mM KCl, 1.2 mM KH_2PO_4, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH

5   7.4) and lysed by sonication in ice-cold hypotonic buffer (20 mM Tris-HCl, 5 mM EDTA, pH 7.7). Large particles and debris were cleared by low speed centrifugation (200 x g, 20 min, 4 °C). Membranes were collected from the supernatant fraction by high speed centrifugation

10 · (32,000 x g, 18 min, 4 °C), washed with ice-cold hypotonic buffer, and collected again by high speed centrifugation (32,000 x g, 18 min, 4 °C). The final membrane pellet was resuspended by sonication into a small volume (~500 μl) of ice-cold binding buffer (10 mM NaCl, 20 mM HEPES, 0.22

15   mM KH_2PO_4, 1.26 mM $CaCl_2$, 0.81 mM $MgSO_4$, pH 7.4). Protein concentration was measured by the Bradford method (19) using Bio-Rad Reagent, with bovine serum albumin as a standard.

20   **Radioligand Binding to Membrane Suspensions**
Membrane suspensions were diluted in binding buffer supplemented with 0.1% bovine serum albumin and 0.1% bacitracin to yield membrane protein concentrations of ~ 0.02 mg/ml for human Y1 receptors, ~ 0.003 mg/ml for CG-

25   13 receptors, and ~ 0.25 mg/ml for SK-N-Be(2) (under these assay conditions, non-specific binding of ^{125}I-PYY to membranes was less than 10%). ^{125}I-PYY and non-labeled peptide competitors were also diluted to desired concentrations in supplemented binding buffer.

30   Individual samples were then prepared in 96-well polypropylene microliter plates by mixing membrane suspensions (200 ul), ^{125}I-PYY (25 ul), and non-labeled peptides or supplemented binding buffer (25 ul). Samples were incubated in a 30 °C water bath with

35   constant shaking for 120 min. Incubations were terminated by filtration over Whatman GF/C filters (pre-coated with 0.5% polyethyleneimine and air-dried before

56

use). Filter-trapped membranes were counted for ^{125}I in a gamma counter. Non-specific binding was defined by 100 nM human NPY. Specific binding in time course and competition studies was typically 80%; most non-specific

5 binding was associated with the filter. Binding data were analyzed using nonlinear regression and statistical techniques available in the GraphPAD InPlot package (San Diego, CA).

10 **Creation Of A Stably Expressing Cell Line**

pcEXV-hY2 DNA was transfected into the 293 human embryonic kidney cell line by the calcium phosphate transfection method. The 293 cells were grown in minimal essential medium (MEM) with Hank's salts, plus 2 mM

15 glutamine, 100 international units of penicillin, streptomycin at 100 ug/ml, and 10% fetal calf serum, in 5% CO_2 at 37°C. Stably transfected cells were selected for two weeks in media containing G-148 (1 mg/ml) and screened for the ability to bind ^{125}I-PYY. Several clones

20 were selected based on preliminary measurements of cell density. One positive clone, designated 293-hY2-10, was chosen for further characterization in binding and functional assays. This clone displayed saturable binding of ^{125}I-porcine PYY in membrane preparations: B_{max}

25 = 880 fmol/mg membrane protein, K_d = 3 pM, (n=3). When incubated with various concentrations of human PYY, it elicited a concentration-dependent inhibition of forskolin-stimulated cAMP accumulation as determined by radioimmunoassay. Clone 293-hY2-10 also elicited a

30 concentration-dependent increase in free intracellular calcium as determined by Fura-2 florescence. The calcium response, which probably reflects mobilization of intracellular calcium stores, was inhibited by pretreatment of cells with pertussis toxin. EC_{50} values

35 for both the cAMP and the calcium response are currently under investigation.

57

pcEXV-hY2 DNA was also transfected into the mouse
embryonic NIH-3T3 cell line using the methods described
above to create another cell line stably expressing human
Y2 receptors. A clone designated N-hY2-5 was selected
5 and characterized as above.

**Tissue Localization and Gene Expression: Reverse
Transcriptase PCR**

Human tissues obtained from National Disease Research
10 Interchange were homogenized and total RNA extracted
using guanidine isothiocyanate/CsCl cushion method. RNA
was treated with DNase to remove any contaminating
genomic DNA. cDNA was prepared from total RNA with
random hexanucleotide primers using the reverse
15 transcriptase Superscript II (BRL, Gaithersburg, MD). An
aliquot of the first strand cDNA (250ng of total RNA) was
amplified in a 50 μl PCR reaction mixture (200μM dNTPs
final concentration) containing 1.2U of Taq polymerase
in the buffer supplied by the manufacturer (Perkin-Elmer
20 Corporation), and 1 μM of primers, using a program
consisting of 30 cycles of 94°C./2', 68°C./2', and
72°C./3', with a pre- and post-incubation of 95°C./5' and
72°C./10', respectively. PCR primers for human Y2 were
designed against the human Y2 sequence in the third
25 intracellular loop and carboxyl terminal regions: 5'-
GGGAGTATTCGCTGATTGAGATCAT-3' (SEQ. I.D. No. 21) and 5'-
GCCTTGAATGTCACGGACACCTC-3' (SEQ. I.D. No. 22),
respectively.

30 The PCR products were run on a 1.5% agarose gel and
transferred to charged nylon membranes (Zetaprobe GT,
BioRad), and analyzed as Southern blots. Hybridization
probes corresponding to the receptor region flanked by
PCR primers were prepared
35 (5'-CTGATGGTAGTGGTCATTTGCAGCTCCAGGACTGACATGGTTCTT-3')
(SEQ. I.D. No. 23) and pre-screened for the absence of
cross-reactivity with human Y1 and Y4 receptor subtypes.

58

Filters were hybridized with the phosphorylated probes
and washed under high stringency. Labeled PCR products
were visualized on X-ray film. Similar PCR and Southern
blot analyses were conducted with primers and probe
5   directed to the housekeeping gene, glyceraldehyde-3-
phosphate dehydrogenase (Clontech, Palo Alto, CA), and
demonstrated that equal amounts of cDNA from the
different tissues were being assayed for Y2 receptor
expression.

10

**Localization of NPY Y2 messenger RNA in the rat central
nervous system**
The distribution of NPY Y2 mRNA in the rat brain was
determined using *in situ* hybridization histochemistry.
15 Male Sprague-Dawley rats were euthanized with CO_2,
decapitated and the brains rapidly removed and frozen in
isopentane. Coronal sections were cut at 11 μm on a
cryostat and thaw-mounted onto poly-L-lysine coated
slides and stored at -80° C until use. Prior to
20 hybridization, tissues were fixed in 4% paraformaldehyde,
treated with 5 mM dithiothreitol, acetylated in 0.1 M
triethanolamine containing 0.25% acetic anhydride,
delipidated with chloroform, and dehydrated in graded
ethanols.

25

The oligonucleotide probes employed to characterize the
distribution of the NPY Y2 mRNA were synthesized using a
Cyclone Plus DNA synthesizer (Milligen/Biosearch) and
gel-purified. The probes used and their sequences are
30 given in Table 7. Probe specificity was established by
performing the *in situ* hybridization protocol described
below on cells transfected with the rat NPY Y2 DNA
(supra), or on nontransfected control cells. In
addition, both sense and antisense probes were employed
35 on cells and rat tissues.

Probes were 3'-end labeled with ^{35}S-dATP (1200 Ci/mmol,

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New England Nuclear, Boston, MA) to a specific activity of 10^9 dpm/μg using terminal deoxynucleotidyl transferase (Boehringer Mannheim; Indianapolis, IN). The radiolabeled probes were purified on Biospin 6

5   chromatography columns (Bio-Rad; Richmond, CA), and diluted in hybridization buffer to a concentration of 1.5 x 10^4 cpm/μl. The hybridization buffer consisted of 50% formamide, 4X sodium citrate buffer (1X SSC = 0.15 M NaCl and 0.015 M sodium citrate), 1X Denhardt's solution (0.2%

10 polyvinylpyrrolidine, 0.2% Ficoll, 0.2% bovine serum albumin), 50 mM dithiothreitol, 0.5 mg/ml salmon sperm DNA, 0.5 mg/ml yeast tRNA, and 10% dextran sulfate. One hundred μl of the diluted probe was applied to each section, which was then covered with a Parafilm

15 coverslip. Hybridization was carried out overnight in humid chambers at 40 to 55°C. The following day the sections were washed in two changes of 2X SSC for one hour at room temperature, in 0.1X SSC for 30 min at 50-60°C, and finally in 0.1X SSC for 30 min at room

20 temperature. Tissues were dehydrated in graded ethanols and apposed to Kodak XAR-5 film for 3 days to 6 weeks at −20°C, then dipped in Kodak NTB3 autoradiography emulsion diluted 1:1 with 0.2% glycerol water. After exposure at 4°C for 2 to 8 weeks, the slides were developed in Kodak

25 D-19 developer, fixed, and counterstained with hematoxylin and eosin.

Functional Assay: Radioimmunoassay of cAMP

Stably transfected cells were seeded into 96-well

30 microliter plates and cultured until confluent. To reduce the potential for receptor desensitization, the serum component of the media was reduced to 1.5% for 4 to 16 hours before the assay. Cells were washed in Hank's buffered saline, or HBS (150 mM NaCl, 20 mM HEPES, 1 mM

35 $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, and 10 mM glucose) supplemented with 0.1% bovine serum albumin plus 5 mM theophylline and pre-equilibrated in the same solution

60

for 20 min at 37 °C in 5% CO_2. Cells were then incubated 5 min with 10 μM forskolin and various concentrations of receptor-selective ligands. The assay was terminated by the removal of HBS and acidification of the cells with 5   100 mM HCl. Intracellular cAMP was extracted and quantified with a modified version of a magnetic bead-based radioimmunoassay (Advanced Magnetics, Cambridge, MA). The final antigen/antibody complex was separated from free ^{125}I-cAMP by vacuum filtration through a PVDF 10   filter in a microliter plate (Millipore, Bedford, MA). Filters were punched and counted for ^{125}I in a Packard gamma counter. Binding data were analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

15

Functional Assay: Intracellular Calcium Mobilization
The intracellular free calcium concentration was measured by microspectroflourometry using the fluorescent indicator dye Fura-2/AM. Stably transfected cells were 20   seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells were washed with HBS and then loaded with 100 μl of Fura-2/AM (10 μM) for 20 to 40 min. After washing with HBS to remove the Fura-2/AM solution, cells were equilibrated in HBS for 10 to 20 min. Cells 25   were then visualized under the 40X objective of a Leitz Fluovert FS microscope and fluorescence emission was determined at 510 nM with excitation wave lengths alternating between 340 nM and 380 nM. Raw fluorescence data were converted to calcium concentrations using 30   standard calcium concentration curves and software analysis techniques.

Reagents
Cell culture media and supplements were from Specialty 35   Media (Lavallette, NJ). Cell culture plates (150 mm) were from Corning (Corning, NY). Cell culture flasks (225 cm^2) and polypropylene microliter plates were from

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Co-star (Cambridge, MA). Porcine ^{125}I-PYY was from New England Nuclear (Boston, MA). NPY and related peptide analogs were from either Bachem California (Torrance, CA) or Peninsula (Belmont, CA). Whatman GF/C filters were
5 Brandel (Gaithersburg, MD). Bio-Rad Reagent was from Bio-Rad (Hercules, CA). Bovine serum albumin and bacitracin were from Sigma (St. Louis. MO). All other materials were reagent grade.

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RESULTS

Isolation of a cDNA clone encoding a human hippocampal Y2 receptor

5 In order to clone a human NPY receptor subtype (Y2), we used an expression cloning strategy in COS-7 cells (20, 21, 22). This strategy was chosen for its extreme sensitivity since it allows detection of a single "receptor positive" cell by direct microscopic
10 autoradiography.

Since the Y2 receptor is described as a presynaptic receptor, it is difficult to locate cell bodies that actually contain this specific mRNA in restricted brain
15 areas. We reasoned that human hippocampus was a good source of mRNA since it contains both a large number of interneurons and has been shown to carry a particularly dense population of Y2 receptors (23, 24, 25, 26). A human hippocampal cDNA library of 2.2 x 10^6 independent
20 recombinants with a 3 kb average insert size was fractionated into 440 pools of ≈5000 independent clones. From the first 200 pools tested, three gave rise to positive cells in the screening assay (#145 ,158 and 189). The last 220 pools tested were all negative.
25

Since both Y1 and Y2 receptor subtypes are expressed in the hippocampus (2), we analyzed the DNA of positive pools by PCR with Y1 specific primers. Pools #145 and #158 turned out to contain cDNAs encoding an Y1 receptor,
30 but pool #189, negative by PCR (data not shown), likely contained a cDNA encoding a human hippocampal NPY receptor that was not Y1. Pool #189 was subdivided in 20 pools of 1000 clones each, and a preliminary pharmacological characterization was run on COS-7 cells
35 transfected with DNA prepared from the secondary pools. This preliminary analysis revealed that a 100 fold excess of cold [Leu^{31}-Pro^{34}]NPY totally inhibited binding of ^{125}I-

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PYY to control COS-7 cells transfected with the Y1 gene.
In contrast, no significant inhibition of binding was
observed when the same experiment was performed on COS-7
cells transfected with secondary pool #189-17 (data not
5   shown). This is consistent with pool #189 containing a
cDNA encoding a human hippocampal Y2 receptor. The sib
selection was therefore pursued on pool #189 until a
single clone was isolated (designated CG-13).

10 The isolated clone carries a 4.2 kb cDNA. This cDNA
contains an open reading frame between nucleotides 1002
and 2147 that encodes a 381 amino acid protein (SEQ. I.D.
No. 2). The unusually long 5' untranslated region could
be involved in the regulation of translation efficiency
15 or mRNA stability. The flanking sequence around the
putative initiation codon conforms to the Kozak consensus
sequence for optimal translation initiation (27, 28).

The hydrophobicity plot displayed seven hydrophobic,
20 putative membrane spanning regions which makes the human
hippocampal Y2 receptor a member of the G-protein coupled
superfamily. The nucleotide and deduced amino acid
sequences are shown in Figure 1 and Figure 2,
respectively.
25

Like most G-protein coupled receptors, the Y2 receptor
contains a consensus sequence for N-linked glycosylation,
in the amino terminus (position 11) involved in the
proper expression of membrane proteins (29). The Y2
30 ·receptor carries two highly conserved cysteine residues
in the first two extracellular loops that are believed to
form a disulfide bond stabilizing the functional protein
structure (30). The ·Y2 receptor shows 7 potential
phosphorylation sites for protein kinase C in positions
35 11, 27, 64, 145, 188, 250 and 340, 2 casein kinase sites
in positions 174 and 358, and 2 cAMP- and cGMP-dependent
protein kinase phosphorylation sites in positions 146 and

64

350.   It should be noted that 7 of those 11 potential
phosphorylation sites are located in intra-cellular loops
1, 2 and 3 as well as in the carboxyl terminus of the
receptor and therefore could play a role in regulating
5 functional characteristics of the Y2 receptor (30). A
potential palmitoylation site is present in the sequence
at the cysteine found in position 326. A large number of
G-protein coupled receptors carry a cysteine in the same
position and O'Dowd et al. have speculated that it plays
10 an important role in the functional coupling of the human
β_2-adrenergic receptor (31).   The formation of this
additional cytosolic loop may influence the mobility of
the receptor across the membrane (32).

15 When compared to the published human Y1 cDNA clone (10,
11) the Y2 sequence shows surprisingly low homology both
at the nucleotide level, 48.1% (Figure 3) and overall
amino acid level, 31% (Figure 4).   The transmembrane
domain identity of the human hippocampal Y2 receptor with
20 other 7 TM receptors is shown in Table 1.   The low TM
identity with other G-protein coupled receptor families,
with other peptide receptors and especially with the Y1
subtype raises the possibility that Y2 receptor subtypes
belong to a new distinct sub-family of 7 TM peptide
25 receptors.   Conversely, NPY receptor subtypes could form
a sub-family where members show unusually low levels of
overall homology. Applicants have also cloned the human
Y4 receptor, and this receptor also exhibits a low degree
of homology with the human Y2 receptor (Table 1).   It is
30 interesting to observe that the mouse orphan receptor
MUSGIR (mouse glucocorticoid induced receptor, 33) shows
the highest TM identity (42%, Table 1) with our human Y2
receptor. The same comparison between human Y1 (or Y4)
and Y2 TM regions only gives a score of 41% identity. If
35 we were comparing the human Y2 receptor sequence with the
human homolog of the MUSGIR receptor, the level of
identity might even be higher.   Therefore the MUSGIR

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receptor could be related to the NPY receptors and bind members of the pancreatic polypeptide ligand family. A full pharmacological evaluation of the human GIR homolog with NPY, PYY and PP related ligands is now underway to
5   verify this hypothesis.

Using the human Y2 probe, northern hybridizations reveal a unique band at 4.3 kb in human brain after a three-day exposure (Figure 16). This is in good agreement with the
10 4.2 kb cDNA that we isolated by expression cloning and indicates that our cDNA clone is full-length. The mRNA encoding the human Y2 receptor is present in significant amounts in amygdala, corpus callosum, hippocampus, and subthalamic nucleus.   A faint band is detectable in
15 caudate nucleus, hypothalamus and substantia nigra.   No signal could be detected in thalamus. It should be noted that Clontech's MTN blot does not carry any mRNA from cortex or brain stem.

20 Southern hybridizations to human genomic DNA followed by high stringency washes (Figure 17) suggest that the human genome contains a single Y2 receptor gene (single band with EcoRI, HindIII, BamHI and PstI). The faint bands at 9 and 12 kb observed with BglII can be explained by the
25 presence of two BglII restriction sites in the coding region of the human Y2 sequence and are also consistent with a single Y2 receptor gene.

Pharmacology of the transiently expressed human Y2
30 **receptor**
The Y2-like pharmacology of CG-13, originally identified by whole cell autoradiographic techniques, was further defined by membrane binding assays. The gene for the human hippocampal Y2 receptor was transiently expressed
35 in COS-7 cells for full pharmacological evaluation. ^{125}I-PYY bound specifically to membranes from COS-7 cells transiently transfected with the CG-13 construct. The

66

time course of specific binding was measured in the presence of 0.06 nM ^{125}I-PYY (Figure 5). The association curve was biphasic, with approximately 55% of the specific binding occurring during an initial rapid phase
5   and 45% following a slower time course. For the rapid phase, the observed association constant (K_{obs}) was 1.28 $\pm$ 0.02 min^{-1} and $t_{1/2}$ was 0.5 min; equilibrium binding was 95% complete within 2 min and 100% complete within 5 min (n = 3). For the slow phase, K_{obs} was 0.02 $\pm$ 0.00 min^{-1}
10 and $t_{1/2}$ was 37 min; equilibrium binding was 90% complete within 120 min, 95% complete within 160 min and 100% complete within 280 min (n = 3). Total equilibrium binding, composed of both phases, was 95% complete within 120 min and 100% complete within 240 min. The biphasic
15 association curve may reflect a complex pattern of receptor surface binding followed by access to deep-seated binding sites, as has been suggested by Schwartz and co-workers for Y2 receptors (34). For comparison, we also measured the time course of binding to human Y1
20 receptors transiently expressed in COS-7 cells (Figure 5). The association curve was monophasic, with a K_{obs} of 0.06 $\pm$ 0.02 min^{-1} and a $t_{1/2}$ of 12 min; equilibrium binding was 95% complete within 51 min and 100% complete within 90 min (n = 3). The different patterns of association
25 for CG-13 and human Y1 receptors suggest novel mechanisms of receptor/ligand interaction.

Saturation binding data for ^{125}I-PYY were fit to a one-site model with an apparent K_d of 0.069 $\pm$ 0.009 nM and an
30 apparent B_{max} of 7.8 $\pm$ 0.4 pmol/mg membrane protein, corresponding to approximately 7.5×10^5 receptors/cell (n = 3; Figure 6). Given that the transfection efficiency was 20-30% (data not shown), the receptor density on transfected cells was probably closer to 3 x
35 10^6/cell. Membranes from mock-transfected cells, when prepared and analyzed in the same way as those from CG-13-transfected cells, displayed no specific binding of

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^{125}I-PYY.   We conclude that the ^{125}I-PYY binding sites
observed under the described conditions were derived from
the CG-13 construct.

5   Y2 receptor recognition is thought to depend primarily
     upon the four C-terminal residues of NPY (Arg^{33}- Gln^{34}-
     Arg^{35}- Tyr^{36}-NH$_2$) preceded by an amphipathic α-helix (M4,
     M5); exchange of Gln^{34} with Pro^{34} is not well tolerated (4,
     5). We therefore chose several C-terminal fragments and
10   C-terminal modified peptides for competition binding
     studies.   The rank order of affinity for selected
     compounds was derived from competitive displacement of
     ^{125}I-PYY (Fig. 7 and Table 3).   The CG-13 receptor was
     compared with two model systems: 1) the cloned human Y1
15   receptor (10, 11) transiently expressed in COS-7 cells,
     and 2) the Y2-like receptor population expressed by human
     SK-N-Be(2) neuroblastoma cells (2, 8).   To our
     knowledge, no models for human Y3 and human PP receptors
     have been described.
20

     CG-13 bound with high affinity to human NPY (K$_i$ = 0.69
     nM) and even more so to human PYY (K$_i$ = 0.39 nM). The K$_i$
     values are in agreement with numerous reports of
     pharmacologically defined Y2 receptors studied in NPY
25   binding and functional assays (2).   The opposite rank
     order was observed with human Y1 receptors, combined with
     stronger receptor/binding interactions (K$_i$ = 0.049 and
     0.085 nM for human NPY and human PYY, respectively).   It
     is interesting in this regard that CG-13 bound ^{125}I-PYY (K$_d$
30   = 0.069 nM) with higher affinity than PYY (K$_i$ = 0.39 nM),
     suggesting that iodination may stabilize the
     receptor/ligand complex.   The human Y1 receptor, in
     contrast, bound both ^{125}I-PYY (K$_d$ = 0.062 + 0.010 nM, n =
     3, data not shown) and PYY (K$_i$ = 0.085 nM) with comparable
35   affinity. The fact that CG-13 and the human Y1 receptor
     bound NPY, PYY and ^{125}I-PYY with different magnitudes and
     rank orders of affinity most likely reflects distinct

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mechanisms of peptide recognition which could potentially be exploited for the development of subtype-selective non-peptide ligands.

5   CG-13 also bound with high affinity to porcine NPY (K_i = 0.86 nM), which differs from human NPY by containing Leu[17] in the PP-fold rather than Met[17]. CG-13 was relatively insensitive to N-terminal deletion of NPY and PYY; the affinity for porcine NPY_{22-36} was only 5-fold less than

10   that for full length porcine NPY. Extreme deletion of α-helical structure was less well tolerated; the affinity for porcine NPY_{26-36} was 240-fold less than that for full length porcine NPY. Human [Leu[31],Pro[34]]NPY and human PP, both having Pro[34] rather than Glu[34], did not bind well (K_i

15   > 300 nM). Hydrolysis of the carboxyl terminal amide to free carboxylic acid, as in NPY free acid, also disrupted binding affinity for CG-13 (K_i > 300 nM). The terminal amide appears to be a common structural requirement for pancreatic polypeptide family/receptor interactions.

20
The competitive displacement data indicate that CG-13 binds PYY with equal or greater affinity than NPY. The C-terminal region of NPY is the primary pharmacophore. CG-13 does not tolerate exchange of Gln[34] with Pro[34], as

25   revealed by low affinity interactions with human [Leu[31],Pro[34]]NPY and human PP. The binding profile, which is shared by SK-N-Be(2) cell receptors but not by human Y1 receptors, is characteristic of the pharmacologically defined Y2 receptor (refs. 2, 8; see also Table 2). The

30   membrane binding studies therefore confirm and extend our assessment that CG-13 encodes a human Y2 receptor.

The pharmacological profile of the human Y2 receptor was further investigated using peptide analogs related to

35   NPY, PYY, and PP (Table 4). CG-13 did not discriminate human and frog analogs of NPY (K_i = 0.74 and 0.87 nM, respectively), human and porcine analogs of NPY_{2-36} (K_i =

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2.0 and 1.2 nM, respectively), human and porcine analogs of [Leu^{31}, Pro^{34}]NPY (K_i > 130 and > 540 nM, respectively), or human NPY and human [Tyr-O-Me^{21}]NPY (K_i = 0.74 and 1.6 nM, respectively). This last derivative

5 was tested based on the proposal that it was selective for central vs. peripheral NPY receptors, with high binding affinity in rat CNS but low potency in rat vas deferens relative to NPY (83). For the receptors under investigation, however, [Tyr-O-Me^{21}]NPY and human NPY

10 yielded highly similar binding profiles. The NPY derivative with greatest selectivity for CG-13 was C2-NPY, a C^2 to C^{27} disulfide-stabilized derivative of NPY with an 8-amino-octanoic linker replacing NPY$_{5-24}$ (K_i = 3.5 nM, $\geq$ 20-fold selective for CG-13 over Y1 and Y4

15 receptors). C2-NPY has been described as a Y2-selective compound (3).

Three additional PYY derivatives yielded distinctive binding profiles. CG-13 bound with highest affinity and

20 greatest selectivity to human PYY$_{3-36}$ (K_i = 0.70 nM, $\geq$ 20-fold selective for CG-13 over Y1 and Y4 receptors). PYY$_{3-36}$ is a major form of PYY-like immunoreactivity in blood and could therefore mediate CG-13-dependent processes *in vivo* (84, 85). Porcine PYY was relatively nonselective

25 and similar in binding affinity to human PYY (K_i = 0.35 nM and 0.36 nM, respectively). Human [Pro^{34}]PYY was lacking in binding affinity for CG-13 (K_i > 310), further supporting the argument that Pro^{34} is disruptive for high affinity peptide binding to the CG-13 receptor.

30

Six additional PP derivatives were investigated. Those peptides which resemble human PP in that they contain Pro^{34} (bovine, rat, avian, and frog PP) displayed no activity in the CG-13 binding assay. High affinity

35 binding was detected only for salmon PP (K_i = 0.17 nM), which is distinguished by containing Gln^{34}. When the C-terminus of human PP was modified to more closely

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resemble human NPY, as in [Ile^{31}, Gln^{34}]PP, the binding
affinity for CG-13 was increased dramatically (K_i = 20
nM). It has been reported previously that [Ile^{31}, Gln^{34}]PP
was more active than PP in Y2 binding assays, while
5   exhibiting decreased potency for putative PP receptors in
rat vas deferens (86).

Several proposed NPY antagonists were analyzed for their
ability to bind to CG-13 receptors. These include PYX-1
10   and PYX-2, C-terminal derivatives of NPY reported to
antagonize NPY-mediated feeding and neurotransmitter
release (87, 88, 89). Neither synthetic peptide bound to
CG-13 with high affinity or selectivity (K_i = 684 for PYX-
1 and K_i > 1000 nM for PYX-2). [D-Trp^{32}]NPY is an NPY
15   derivative reported to regulate feeding behavior when
injected into the hypothalamus of rats (90); this analog
was inactive in the CG-13 binding assay. Another
inactive compound was NPY$_{1-24}$ amide, a peptide reported to
antagonize NPY in the rat vas deferens (83).
20

Human tissue Y2 receptor macrolocalization: PCR
Human Y2 mRNA was detected by PCR techniques in a broad
range of human tissues (Table 5). Relatively intense
hybridization signals were detected in total brain,
25   thoracic artery, coronary artery, and penis, with more
moderate levels in frontal brain, ventricle, mesentery,
stomach and ileum. Relatively low levels were detected
in nasal mucosa and pancreas. Several other tissues were
negative for Y2 mRNA as measured by this technique,
30   including atrium, liver, and uterus.

**Cloning and Expression of Two Isoforms of the Rat NPY/PYY
(Y2) Receptor**
Two rat genomic clones homologous to the human Y2
35   receptor were isolated, termed rs5a (Figure 8) and rs26a
(Figure 9). The nucleotide sequence of rs5a is 86.5%
identical in the coding region to that of the human Y2

71

receptor (Figure 10), and can encode a 381 amino acid protein with 94.5% identity to the human Y2 amino acid sequence (Figure 11). In the putative transmembrane domains (TMs), the protein predicted by rs5a exhibits

5 98.2% amino acid identity with the human Y2 receptor (Figure 11). This high degree of primary sequence identity is often observed for species homologues, and strongly suggests that the receptor encoded by rs5a is the rat Y2 receptor. However, even a single amino acid

10 substitution can influence the functional properties of a receptor; thus, even species homologues exhibiting a high level of sequence identity may display different pharmacological properties (infra), underscoring the importance of obtaining both rat and human receptors for

15 use in drug development.

Sequence analysis of the second genomic clone revealed that rs26a also encoded a full-length rat Y2 receptor; however, rs26a contains two nucleotide changes when

20 compared with the sequence of rs5a. Both nucleotide changes result in amino acid substitutions in the predicted rat Y2 receptor protein. With two (2) amino acid changes, the protein encoded by rs26a is 99.7% identical to that of rs5a. Compared with the human Y2

25 receptor, the nucleotide sequence identity of rs26a is 85.2% and the amino acid sequence identity is 98.2%. This clone therefore encodes an isoform of the rat Y2 receptor distinct from that encoded by rs5a. The locations of the amino acid substitutions (N-terminus and

30 5/6 loop; see Figure 3) suggest that they could potentially influence receptor function. The Y2 receptors encoded by rs5a and rs26a are likely to represent allelic variants at the same gene locus; however, rs26a could represent a second rat Y2 gene.

35 Accordingly, we have designated the isoform encoded by rs5a as the rat Y2a receptor, and designated the isoform encoded by rs26a as the rat Y2b receptor.

72

The primary sequences of rat and human Y2 receptors, while highly related, show distinct patterns of sequence motifs for N-linked glycosylation, N-myristoylation, and
5 protein phosphorylation.   For example, the rat Y2a differs from the rat Y2b in that it contains an additional site for phosphorylation by protein kinase C. Further, the human Y2 differs from both rat Y2 isoforms in containing two additional sites for N-linked
10 glycosylation, two additional sites for cAMP- and cGMP-dependent protein phosphorylation, an additional site for casein kinase II phosphorylation, one additional site for protein kinase C phosphorylation, and two fewer sites for N-myristoylation. These sites could mediate differences
15 in the function or regulation of the three receptors. The isolation of two rat homologues of the Y2 receptor provides the means to compare the pharmacological properties of the rat and human Y2 receptors (see below) in relation to their observed differences in primary
20 structures.   These data will be critical to the design and testing of human therapeutic agents acting at these sites.

Binding Studies with Rat Y2 Homologs
25 The DNA corresponding to the rat Y2a homolog was transiently expressed in COS-7 cells for membrane binding studies. The binding of ^{125}I-PYY to the rat Y2a receptor was saturable over a radioligand concentration of 0.5 pM to 2.5 nM.   Binding data were fit to a one-site model
30 with an apparent K_d = 0.26 nM and a receptor density of 5100 fmol/mg membrane protein. As determined by using peptide analogs within the pancreatic polypeptide family, the rat Y2a pharmacological profile resembles that for the human Y2 receptor (Table 6). Each receptor analog
35 is relatively tolerant of N-terminal ligand deletion (the human apparently more so than the rat) and intolerant of any peptide containing Pro^{34} or a modified C-terminus (as

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in NPY free acid or [D-Trp^{32}]NPY).

The rat Y2b clone, which differs from rat Y2a by two amino acid changes one in the N-terminal tail (from Leu^{20} to Phe^{20}) and another in the third intracellular loop (from Thr^{266} to Met^{266}), has been subjected only to a preliminary investigation. Membranes from COS-7 cells transiently transfected with the rat Y2b receptor were incubated with 0.08 nM ^{125}I-PYY and analyzed for specific binding after incubation at 30 °C for 120 min. Membranes from transfected cells bound 310 fmol ^{125}I-PYY/ mg membrane protein, whereas membranes from mock-transfected cells (receiving vector without receptor cDNA insert) bound only 3 fmol ^{125}I-PYY/ mg membrane protein. It remains to be determined whether there exist any pharmacological or functional differences between the ratY2a and rat Y2b receptors.

Localization of NPY Y2 messenger RNA in the rat central nervous system

In control experiments, hybridization signals for rat NPY Y2 mRNA were seen only with the antisense probes (probe sequences shown in Table 7), and only over cells which had been transfected with the rat Y2 DNA (Figure 18). The probes were designed to recognize both rat Y2a and rat Y2b. Neither mock transfected cells nor cells transfected with rat NPY Y1 mRNA exhibited hybridization signals. On rat brain sections, no hybridization signals were obtained with the sense probes, only with the antisense probes.

The distribution of NPY Y2 mRNA observed in coronal sections through the rostrocaudal extent of rat brain is shown in Figure 12 and Table 8. Hybridization signals were seen over many areas of the rat brain (Figure 12), which, at the microscopic level, were confined to the cytoplasm of neuronal profiles (data not shown). In the

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telencephalon, the most intense hybridization signals were observed over the CA3 region of the hippocampus (Figure 12B-E) and over the anteroventral aspect of the medial nucleus of the amygdala (Figure 12C, D). Less

5 intense signals were found over the olfactory tubercle, the lateral septal nucleus (Figure 12A), and over the basomedial nucleus and posteromedial cortical nucleus of the amygdala (Figure 12D, E). Scattered neurons with hybridization signal were also seen in the central

10 amygdaloid nucleus. In cortex, silver grains were seen over large neurons in the piriform region.

Among diencephalic structures, the arcuate nucleus of the hypothalamus exhibited the most intense hybridization

15 signal for NPY Y2 mRNA (Figure 12D, E). In this area, most of the neurons appeared to be labelled, and many neurons were also labelled in the region of the tuber cinereum lateral to the arcuate nucleus. In addition, both the dorsomedial and ventromedial hypothalamic nuclei

20 contained appreciable hybridization signals over subpopulations of neurons (Figure 12C, D). In the dorsal and ventral premammillary nuclei, hybridization signal was seen over many neurons (Figure 12E). In the thalamus, neurons in the centromedial nucleus were

25 labelled (Figure 12C, D), while a smaller, less intensely labelled group of cells was visible in the paraventricular nucleus (Figure 12D).

In the mesencephalon, medulla, and pons, few structures

30 were labelled with the antisense oligonucleotide probe. Those exhibiting a moderate level of hybridization signal were the dorsal and caudal linear raphe (Figure 12F), the pontine nucleus, and the posterior dorsal tegmental nucleus (Figure 12G). In the spinal cord, labelling was

35 observed over scattered large neurons in lamina 9 (Figure 12H). Silver grains were also found over a few large neurons in the dorsal root ganglion.

75

Receptor/G protein Interactions: Effects of Guanine Nucleotides

For a given G protein-coupled receptor, a portion of the receptor population in a membrane homogenate typically

5   exists in the high affinity ligand binding state as a receptor/G protein complex. The binding of GTP or a non-hydrolyzable analog to the G protein causes a conformational change in the receptor which favors a low affinity ligand/binding state (110). We investigated

10 whether the non-hydrolyzable GTP analog, Gpp(NH)p, would alter the binding of human NPY or ^{125}I-PYY to Y2 receptors transiently expressed in COS-7 cells. The competition curve produced by human NPY was evaluated in the absence and presence of 100 μM Gpp(NH)p. The human Y2 receptor

15 was relatively insensitive to the Gpp(NH)p compared to the rat Y2a receptor (Figure 13). The IC_{50} for human NPY binding to the human Y2 receptor was increased from 2.2 nM to 3.3 nM; specific binding of ^{125}I-PYY was decreased by only 4% (n = 5). The IC_{50} for human NPY binding to the

20 rat Y2a receptor was altered very little (from 0.7 nM to 1.2 nM, n = 2); specific binding of ^{125}I-PYY, however, was decreased by 23% (n = 2). A similar pattern of sensitivity to Gpp(NH)p was reported for ^{125}I-PYY binding to rat brain (91). The difference between the rat and

25 human Y2 receptor clones could be explained by several factors, including 1) the types of G proteins available in COS-7 cells, 2) the level of receptor reserve in COS-7 cells (note that human Y2 receptor density was greater than that of the rat Y2a receptor), and 3) the efficiency

30 of receptor/G protein coupling (92; 93).

Stable Expression Systems: Characterization in Binding Assays

Untransfected 293 and NIH-3T3 cells were pre-screened for

35 specific ^{125}I-PYY binding and found to be negative (data not shown). After co-transfection with the human Y2 cDNA plus a G-418-resistant gene and selection with G-418,

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surviving colonies were screened for specific binding of ^{125}I-PYY. Two positive clones were identified and isolated for further study (293 clone #10 and NIH-3T3 clone #5). The binding of ^{125}I-PYY to membranes from the 293 stable

5 clone was saturable over a radioligand concentration range of 0.5 pM to 2.5 nM. Binding data were fit to a one-site binding model with an apparent K_d of 3 ± 1 pM and a receptor density of 880 ± 50 fmol/mg membrane protein (mean $\pm$ s.e.m., n = 3). Membranes from stably

10 transfected NIH-3T3 cells displayed similar binding properties, with an apparent K_d of 8 ± 2 pM and a receptor density of 160 ± 60 fmol/mg membrane protein (mean $\pm$ s.e.m., n = 2). Membranes from both stable clones were incubated with 0.08 nM ^{125}I-PYY in the presence or absence

15 of 100 μM Gpp(NH)p. Specific binding of ^{125}I-PYY to Y2 receptors in 293 cell membranes was reduced 32% in the presence of the guanine nucleotide, whereas specific binding to Y2 receptors in NIH-3T3 cell membranes was reduced only 6% under the same conditions. The data

20 serve to emphasize that the receptor/G protein interactions for a given receptor clone can vary depending upon the resident G proteins in the host cell line (93). Additional factors such as receptor density and receptor reserve can also play a role (92).

25

Functional Assay: cAMP

Activation of all Y-type receptors described thus far is thought to involve coupling to G-proteins which are inhibitory for adenylate cyclase activity (G_i or G_o) (1).

30 Based on these prior observations, we investigated the ability of PYY to inhibit forskolin-stimulated cAMP accumulation in 293 cells stably expressing the human Y2 receptor. Incubation of intact cells with 10 μM forskolin produced a 10-fold increase in cAMP

35 accumulation over a 5 minute period, as determined by radioimmunoassay. Simultaneous incubation with human PYY decreased the forskolin-stimulated cAMP accumulation by

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71% in stably transfected 293 cells (Figure 14) but not
in untransfected cells (data not shown). The NPY-
mediated response was concentration-dependent (EC_{50} = 0.25
nM). We conclude that human Y2 receptor activation can
5   result in decreased cAMP accumulation, very likely
through inhibition of adenylate cyclase activity. Similar
results were obtained for NIH-3T3 cells stably
transfected with the human Y2 receptor, in which human
NPY decreased forskolin-stimulated cAMP accumulation by
10 50% in transfected cells with an EC_{50} of 0.21 nM (Figure
14).

Peptides selected for their ability to bind to the
transiently expressed human Y2 receptor were further
15 investigated for functional activity using stably
transfected 293 cells (Table 9). All peptides with
measurable binding affinity were able to mimic the
effects of PYY on cAMP accumulation. EC_{50} values were
generally within a 10-fold range of K_i values, often lower
20 in magnitude (Table 9). We also investigated the
functional activity of the reported feeding behavior
modulator [D-Trp^{32}]NPY. Consistent with this peptide's
low binding affinity for the human Y2 receptor, we
detected no functional activity at concentrations up to
25 0.3 μM, or when tested at 0.3 μM for antagonism of the
functional response (data not shown). The reported NPY
receptor antagonists PYX-1 and PYX-2 were also inactive
when tested under the same paradigm.

30 **Functional Assay: Intracellular Calcium Mobilization**
The intracellular free calcium concentration was
increased in 293 cells stably transfected with the human
Y2 receptor after application of 1 μM human PYY (Δ [Ca^{2+}]$_i$
= 80 nM; Figure 15). The PYY-mediated response was
35 concentration-dependent, with EC_{50} = 39 nM, n = 2 (Figure
15). PYY-induced calcium mobilization was relatively
maintained in the presence of 1 mM extracellular EGTA (Δ

$[Ca^{2+}]_i$ = 64 nM for 1 μM human PYY), suggesting that
intracellular calcium stores are the primary source of
the transient calcium flux. Pretreatment with pertussis
toxin (100 ng/ml for 24 hours) decreased the response to
5   300 nM human PYY by 93%, thereby supporting a G protein-
linked signal transduction pathway. Untransfected 293
cells did not respond to human PYY (data not shown). The
calcium mobilization assay provides a second pathway
through which Y2 receptor activation can be measured.
10

DISCUSSION

Attempts to isolate the NPY Y2 receptor subtype based on
15   sequence homology with the Y1 receptor have not been
successful so far. Therefore, we chose an expression
cloning approach where a functional receptor is actually
detected with exquisite sensitivity on the surface of
transfected cells, using a highly specific iodinated
20   ligand. Using this strategy, we have identified a human
cDNA encoding the pharmacologically defined Y2 receptor.
The fact that we had to screen 2.2 x 10^6 independent
clones with a 3 kb average insert size to find one clone
reveals either a very strong bias against Y2 cDNA cloning
25   in the cDNA library construction procedure, or the Y2
mRNA is expressed at very low levels in human hippocampal
tissue. The longest reading frame in the cDNA encodes
a 381 amino acid protein with an estimated molecular
weight of 42 kD. Given the fact that there is an N-
30   linked glycosylation site in the amino terminus, the
apparent molecular weight could be slightly higher and in
good agreement with published data on the molecular
weight of the human hippocampal Y2 receptor at 50 kD
(36). The Y2 receptor carries a large number of
35   potential phosphorylation sites which could be involved
in the regulation of its functional characteristics.

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The nucleotide and amino acid sequence analysis both
reveal low identity levels with all 7-TM receptors
including the human Y1 and Y4 receptors. The highest
transmembrane amino acid identity is found with the mouse
5   MUSGIR receptor. A pharmacological profile on the human
GIR homolog will be established with NPY, PYY and
pancreatic polypeptide related ligands to find out if
this   orphan   receptor   belongs   to   the   same
pharmacologically defined neuropeptide Y receptor sub-
10 family. The human Y2 receptor shares very low amino acid
identity with the previously cloned human Y1 receptor
(31% overall and 41% in transmembrane regions). The
human Y2 receptor also displays a unique pharmacological
profile and a unique time course of association with ^{125}I-
15 PYY.   The   dramatic   differences   in   sequence   and
pharmacological profile between the human Y1 and Y2
receptors suggest that they might be encoded by two
unrelated genes whose products have evolved into binding
the same family of ligands. Conversely, they could have
20 diverged from a common ancestor very early in evolution
and undergone multiple mutations leading to distinct
pharmacological characteristics.

Northern analysis reveals a 4.3 kb band in human brain
25 and demonstrates that our 4.2 kb Y2 cDNA is full-length.
Southern analyses are consistent with the human genome
containing a single Y2 receptor gene.

The pharmacological binding profile established in our
30 initial characterization served primarily to establish
the CG-13 as a human Y2 receptor. The additional data
included here reflect an increased understanding of
receptor ligand/interactions. We now know, for example,
that C2-NPY and PYY$_{3-36}$ can be used to compete for Y2
35 receptor sites with greater affinity and selectivity than
the C-terminal fragments of NPY originally described. We
also know that certain peptides which are thought to

80

antagonize NPY-dependent effects, such as [D-Trp^{32}]NPY, PYX-1, and PYX-2, are unable to compete for binding of the human Y2 receptor clone described here. Our evidence does not therefore support the cloned Y2 receptor as the
5   molecular target of these particular peptides _in vivo_ or _in vitro_.

Human Y2 receptor mRNA was detected by PCR techniques in a broad range of human tissues (Table 5). Relatively
10 intense hybridization signals were detected in total brain, thoracic artery, coronary artery, and penis, with more moderate levels in frontal brain, ventricle, and mesentery. This distribution is consistent with evidence for Y2 receptor localization and receptor-dependent
15 effects in CNS, cardiovascular, and reproductive physiology (94). Moderate hybridization signals were also detected in stomach and ileum, consistent with evidence for Y2-mediated effects on chief cell cAMP accumulation (95) and also intestinal electrolyte flux
20 (61; 96). Relatively low levels were detected in nasal mucosa and pancreas, two tissues in which Y2-like receptors have been reported to regulate vasoconstriction and pancreatic secretion, respectively (97, 98, 99). A more definitive localization of the Y2 receptor mRNA and
25 receptor expression (i.e., whether on neurons, enterocytes, vascular smooth muscle cells, etc.) is attainable through _in situ_ hybridization and receptor autoradiography techniques.

30 The distribution of NPY Y2 mRNA described here in rat brain has a number of potential implications, and raises a number of important questions. Among these are; 1) how does the distribution of this mRNA correlate with that of NPY itself; 2) how does the Y2 mRNA distribution relate
35 to the putative autoradiographic localization of Y2 receptors described by previous investigators; and 3) what are the functional implications of the Y2 mRNA

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distribution?

Correlation with NPY immunoreactivity

Neuropeptide Y is one of the most abundant and widely
5 distributed peptides in the mammalian brain (100). In
some areas, NPY Y2 mRNA appears to be co-distributed with
NPY-immunoreactive (NPYir) neurons, although
colocalization in the same neuron(s) remains to be
established. In both the arcuate nucleus of the
10 hypothalamus and the medial nucleus of the amygdala, the
distribution of Y2 mRNA overlaps with the distribution of
NPYir neurons demonstrated by immunocytochemical studies
(100, 101). In addition, both areas contain moderate
plexuses of NPYir axons. These observations leave open
15 the question of presynaptic/postsynaptic nature of the Y2
receptor. In most other areas of the brain, the Y2 mRNA
does not appear to be co-distributed with NPYir neurons,
but instead correlates better with the distribution of
NPYir terminal fields, suggesting a postsynaptic
20 localization.

Comparison with receptor autoradiography

A number of investigators have described the distribution
of NPY receptors based on the autoradiographic
25 localization of radiolabelled NPY ligands, among them
$[^{125}I]$NPY and $[^{125}I]$peptide YY (PYY), in combination with
subtype-selective displacers. The Y2 receptor has been
localized by combining $[^{125}I]$PYY with the Y2-selective mask
NPY_{13-36} (94). The results of such studies suggest that
30 the Y2 receptor is widely distributed in rat brain, being
most abundant in the hippocampus, olfactory bulb, and
hypothalamus. We have seen no NPY Y2 mRNA in the
olfactory bulb, but both hippocampus and hypothalamus
contain Y2 mRNA. However, the pharmacological
35 characterization of NPY receptor subtypes is incomplete
at present, and some of the Y2-like binding may be
attributable to the so-called atypical Y1 receptor, or to

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other undiscovered NPY receptor subtypes.

Our *in situ* results suggest that the receptor autoradiographic characterization of the Y2 receptor is likely to be accurate for some areas. The projection
5   fields of neurons containing the Y2 mRNA are important in this respect. Thus the pyramidal neurons of the CA3 region of the hippocampus, which contain relatively intense Y2 hybridization signals, project in a topographic fashion to the lateral septum (102), an area
10   which supposedly contains a high proportion of Y2 receptors (103, 23, 94). Similarly, the olfactory bulb appears to contain mainly NPY receptors of the Y2 subtype. While there is no Y2 mRNA in the olfactory bulb, the piriform cortex contains many neurons which are
15   labelled with the Y2 antisense probe, and provides a major source of olfactory bulb afferents. The localization of NPY Y2 mRNA in the arcuate nucleus of the hypothalamus is particularly interesting, as NPYir neurons in this nucleus provide the NPY innervation of
20   much of the hypothalamus, including the paraventricular and dorsomedial nuclei (104, 105). It is unclear at present which receptor subtype(s) predominate in the paraventricular nucleus, but based on our results with the Y2 mRNA, and those of Mikkelsen and colleagues with
25   the Y1 mRNA (106, 107), both Y1 and Y2 should be present. Similar arguments can be pursued for most of the regions which contain Y2 mRNA, however a definitive profile of Y2 receptor localization awaits the introduction of Y2 selective ligands.
30

Functional considerations

Neuropeptide Y is involved in a number of physiological functions, including the regulation of food intake, neuronal excitability, cardiovascular regulation, and
35   circadian rhythms. With regard to food intake, the paraventricular nucleus of the hypothalamus is one site which has been intensively investigated, and has been

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demonstrated to be a prominent locus of action for the orexigenic effects of NPY. The localization of NPY Y2 mRNA in the arcuate nucleus, and the projections of the arcuate to the paraventricular nucleus, suggest the

5   involvement of this receptor in feeding.

In the hippocampus, NPY immunoreactivity is found mainly in interneurons which innervate pyramidal cells. Here, NPY has been demonstrated to reduce synaptic excitation

10 in areas CA1 and CA3. This has been assumed to be mediated by a Y2 receptor (108), as C-terminal fragments of NPY are effective in the assay. The localization of Y2 mRNA in pyramidal cells of CA3 indicates that this receptor may be involved in the termination of convulsive

15 activity, such as in epilepsy.

The rat Y2a and Y2b receptor analogs represent essential tools for pharmaceutical drug development. Drug candidates screened primarily against human receptors

20 must also be characterized at the rat (or other relevant species analog) so that data generated from _in vivo_ models can be interpreted accurately. While the current panel of peptides revealed no major differences in pharmacological profile between the human Y2 and rat Y2a

25 receptor analogs, even a single amino acid difference between receptors displaying high sequence similarity could have dramatic effects on ligand binding affinity (109). The rat Y2b receptor represents an additional opportunity to evaluate species-dependent differences in

30 ligand binding. It remains to be determined whether the rat Y2b receptor plays a singular role in rat Y2 receptor pharmacology, due either to unique ligand binding properties or to distinctive localization patterns.

35 We established functional assays for human Y2 receptor activation in both 293 and NIH-3T3 cells based on receptor-dependent inhibition of forskolin-stimulated

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cAMP accumulation (Table 9). The EC_{50} values for peptides in these assays were generally smaller than the corresponding K_i values, suggesting that receptor activation occurs through a high affinity state of the
5 receptor which is not predominantly represented under the conditions of the binding assay. Such a scenario would be consistent with the weak effect of Gpp(NH)p on radioligand binding to the human Y2 receptor in membrane homogenates.
10

Our characterization of the Y2 receptor stably expressed in 293 cells also shows definitively that the Y2 receptor can couple simultaneously to both cAMP regulation and calcium mobilization in a single cell type. The calcium
15 mobilization in 293 cells, at least, appears to occur through a pertussis toxin-sensitive G protein. The EC_{50} for the human PYY-mediated calcium response is significantly larger than that for the cAMP response in the same host cell (39 nM vs. 0.31 nM, respectively),
20 suggesting that calcium mobilization requires promiscuous coupling of the receptor to a G protein other than that involved in cyclase regulation. The exact identities of the G proteins mediating these receptor activation events, whether G_i, G_o, G_z, or another type, remain to be
25 determined.

We now have several Y2 receptor expression systems from which to choose, each uniquely suited to different uses. The transient expression system in COS-7, for example,
30 allows us to generate sufficient quantities of membranes for routine structure/activity relationship measurements. We can also produce mutant receptors by site-directed mutagenesis or related enzymatic techniques and express them transiently in COS-7 for a comparison of
35 pharmacological properties with those of the wild-type receptor. In this way, we can gain insight into receptor binding pockets, ligand binding domains, and mechanisms

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of activation. The stable expression system in 293 and NIH-3T3 cells offers the convenience of a single transfection followed by routine passaging techniques. The stable expression system also offers the opportunity

5   to select for optimum receptor expression levels, G protein populations, and signal transduction pathways, all of which are critical elements for <u>in vitro</u> functional assays. Such assays can be used to determine agonist or antagonist activity in receptor-selective

10 compounds, thereby generating critical information for drug design.

The expression cloning of a human Y2 receptor allows, for the first time, the ability to develop NPY-receptor

15 subtype specific drugs and represents a major advance in our ability to analyze · NPY-mediated physiological processes. Pharmacologically defined Y2 receptors have a widespread anatomical distribution (2). They represent the predominant NPY receptor in brain, with the highest

20 density in hippocampus and relatively high expression in almost all other areas including olfactory bulb, basal ganglia, amygdaloid complex, thalamic and hypothalamic nuclei, pituitary, pineal gland, cerebellum, and brainstem. This distribution is consistent with northern

25 blot analysis, which shows that the Y2 MRNA is present in amygdala, candate nucleus, corpus callosum, hippocampus, hypothalamus, substantia nigra and subthalamic nucleus. Peripheral localization includes sympathetic neurons, dorsal root ganglia, stomach chief cells, intestinal

30 enterocytes, kidney proximal tubule, trachea, and vascular smooth muscle. Y2 receptors are therefore in a position to potentially regulate a variety of physiological functions including cognitive enhancement, circadian rhythm, EEG synchronization, body temperature,

35 blood pressure, locomotor activity, neuroendocrine release, sympathetic activation, sensory transmission, gastrointestinal function, intestinal secretion, renal

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absorption, and cardiovascular function (1, 2).

Y2 receptors are attractive targets for drug design (1).
Y2 receptor regulation may be useful in the treatment of
5   several pathophysiological conditions (1, 2) including
memory loss (111), epileptic seizure (72), pain (64),
depression, hypertension, locomotor problems, sleep
disturbances, eating disorders, sexual/reproductive
disorders, nasal congestion (97), and diarrhea (112). A
10 rigorous investigation of Y2-related pathophysiology has
been hindered by the absence of suitable non-peptide
ligands. The chemical synthesis of subtype selective
agonists and antagonists as potential drug candidates
will be greatly accelerated by screening against a
15 homogeneous population of cloned human Y2 receptors. As
more specific pharmacological tools become available for
probing receptor function, additional therapeutic
indications are likely to be discovered.

20 We do not know whether the human and rat Y2 receptors we
have discovered account for all of the pharmacological Y2
receptors so far described, or whether the Y2 receptor
population is further divided into distinct receptor
subtypes. Indeed, there is some suggestion of receptor
25 heterogeneity within the Y2 receptor population (2).
These are issues which can now be resolved using
nucleotide sequence from the human Y2 receptor as the
basis for in situ localization, anti-sense strategies,
homology cloning, and related techniques. Such
30 approaches will enable us to investigate the existence of
potentially novel NPY receptor subtypes, in humans and
other species, with additional pharmacologic and
therapeutic significance.

35

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**TABLE 1: % aminoacid TM identity of the NPY-2 receptor
with other 7 TM Receptors**

m MUSGIR	42	h Y-1	41	
		h Y-4	41	
h 5HT1A	28	h Adenosine A2b	28	h
Substance K	33			
h 5HT2	31	h Adenosine A1	29	.h
Substance P	32			
h α-adrenergic-1b	34	h Dopamine D1	31	h
Neurokinin-3	33			
h α-adrenergic-2a	34	h Dopamine D2	32	h
Interleukin-8	33			
h β-adrenergic-1	35	bov Hist H1	25	h
Angiotensin$_1$	33			
		h Hist H2	28	h
Angiotensin$_2$	27			
				m
Thyrotropin				
releasing hormone	27			
				h
Bradykinin	25			
				r
mas oncogene	20			

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TABLE 2: Pharmacologically defined receptors for NPY and related pancreatic polypeptides.

Rank orders of affinity are based on published reports of binding and functional data (M9, M24, M3, M10). Missing peptides in the series reflect a lack of published information.

Receptor	Affinity $(-pK_1$ or $-pEC_{50})$					
	11 to 10	10 to 9	9 to 8	8 to 7	7 to 6	< 6
Y1	NPY PYY $[Leu^{31},Pro^{34}]NPY$		NPY_{2-36}	NPY_{13-36}	PP	
Y2		PYY NPY NPY_{2-36}	NPY_{13-36}			$[Leu^{31},Pro^{34}]NPY$ PP
Y3		NPY	$[Pro^{34}]NPY$	NPY_{13-36} PP		PYY
PP	PP		$[Leu^{31},Pro^{34}]NPY$			NPY

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TABLE 3: Pharmacological profile of the CG-13 receptor.

Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing CG-13 receptors. Peptides were tested at concentrations ranging from 0.001 nM to 100 nM. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the equation, $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ^{125}I-PYY concentration and K_d is the equilibrium dissociation constant of ^{125}I-PYY. The data shown are representative of at least two independent experiments.

Competitor	Human Y1, K_i (nM)	CG-13, K_i (nM)	SK-N-Be(2), K_i (nM)
human PYY	0.085 ± 0.021	0.39 ± 0.05	0.11 ± 0.02
human NPY	0.049 ± 0.009	0.69 ± 0.14	0.13 ± 0.02
porcine NPY$_{2\text{-}36}$	1.4 ± 0.2	0.78 ± 0.13	0.41 ± 0.09
porcine NPY	0.049 ± 0.001	0.86 ± 0.13	0.28 ± 0.04
porcine PYY$_{13\text{-}36}$	32 ± 7	1.5 ± 0.2	0.86 ± 0.14
porcine NPY$_{18\text{-}36}$	28 ± 5	1.5 ± 0.2	2.1 ± 0.5
porcine NPY$_{13\text{-}36}$	51 ± 16	2.4 ± 0.4	1.8 ± 0.4
porcine NPY$_{20\text{-}36}$	62 ± 6	3.4 ± 0.3	3.1 ± 0.6
porcine NPY$_{16\text{-}36}$	45 ± 4	3.8 ± 0.7	5.0 ± 0.5
porcine NPY$_{22\text{-}36}$	170 ± 30	4.6 ± 0.1	3.2 ± 0.6

Table 3 continued			
Competitor	Human Y1, K_1 (nM)	CG-13, K_1	SK-N-Be(2) K_1 (nM)
porcine NPY $_{26\text{-}36}$	> 300	210 ± 60	70 ± 7
human NPY free acid	> 300	> 300	280 ± 120
human PP	200 ± 70	> 300	> 300
human [Leu^{31},Pro^{34}] NPY	0.13 ± 0.02	> 300	> 300

TABLE 4: **Extended pharmacological binding profile of the human Y2 receptor vs. other Y-type receptors cloned from human.**

Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing human Y1, human Y2, and human Y4 receptors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the equation Chang-Prusoff equation, $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ^{125}I-PYY concentration and K_d is the equilibrium dissociation constant of ^{125}I-PYY. Any peptide not included in initial characterization shown in previous tables is referred to as a "new peptide". Data shown are representative of at least two independent experiments.

Peptide	Y1	Y2	Y4	Comments
NPY, human	0.08	0.74	2.2	
NPY, porcine	0.07	0.81	1.1	
NPY, frog (melanostatin)	0.07	0.87	1.2	new peptide
O-Me-Tyr^{21}-NPY, human	0.12	1.6	6.1	new peptide
C2-NPY, porcine	73	3.5	120	new peptide
NPY$_{2-36}$, human	3.6	2.0	16	new peptide
NPY$_{2-36}$, porcine	2.4	1.2	5.6	
NPY$_{13-36}$, porcine	70	2.5	38	
NPY$_{16-36}$, porcine	41	3.6	54	
NPY$_{18-36}$, porcine	70	4.2	> 300	
NPY$_{20-36}$, porcine	63	3.6	120	
NPY$_{22-36}$, porcine	> 1000	18	> 990	
NPY$_{26-36}$, porcine	> 1000	380	300 .	

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Table 4 continued				
Peptide	Y1	Y2	Y4	Comments
[Leu^{31}, Pro^{34}]NPY, human	0.15	> 130	1.1	
[Leu^{31}, Pro^{34}]NPY, porcine	0.15	> 540	1.5	new peptide
NPY free acid, human	490	> 1000	> 1000	
NPY$_{1-24}$ amide, human	> 1000	> 1000	> 1000	new peptide
[D-Trp^{32}]NPY, human	> 1000	> 1000	> 1000	new peptide
PYY, human	0.19	0.36	0.87	
PYY, porcine	0.14	0.35	1.3	new peptide
PYY$_{3-36}$, human	45	0.70	14	new peptide
PYY$_{13-36}$, porcine	33	1.5	46	
[Pro^{34}]PYY, human	0.14	> 310	0.12	new peptide
PP, human	77	> 1000	0.06	
PP, bovine	240	> 830	0.05	new peptide
PP, rat	460	> 1000	0.18	new peptide
PP, avian	400	> 1000	7.0	new peptide
PP, frog	98	> 1000	61	new peptide
PP, salmon	0.20	0.17	3.2	new peptide

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Table 4 continued				
Peptide	Y1	**Y2**	Y3	Comments
[Ile^{31}, Gln^{34}]PP, human	> 86	**20**	0.09	new peptide
PYX-1	507	**684**	794	new peptide
PYX-2	> 1000	**> 1000**	> 1000	new peptide

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TABLE 5:   Macrolocalization of human Y2 receptor mRNA in human tissues by PCR.

Localization data reflect PCR-based amplification of human Y2 cDNA derived from mRNA extracts of human tissues. Southern blots of the PCR products were prepared and hybridized with ^{32}P-labeled oligonucleotide probes selective for Y-type receptor subtypes. The labeled products were recorded on X-ray film and the relative signal density was determined by visual inspection. In this rating scheme, + = faint signal, + + = moderate signal,

+ + + = intense signal.

Human tissues	Human Y2 PCR Product
total brain	+ + +
frontal brain	+ +
ventricle (heart)	+ +
atrium (heart)	(-)
thoracic aorta	+ + +
coronary artery	+ + ½
nasal mucosa	+
mesentery	+ +
stomach	+ +
ileum	+ +
pancreas	+
liver	(-)
kidney	+
bladder	+ ½
penis	+ + +
testes	not determined
uterus (endometrium)	(-)
uterus (myometrium)	(-)

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TABLE 6: Peptide binding profile .of the rat Y2a receptor vs. the human Y2 receptor.

Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing rat Y2a and human Y2 receptors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis ·and converted to K_i values according to the equation Chang-Prusoff equation, $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ^{125}I-PYY concentration and K_d is the equilibrium dissociation constant of ^{125}I-PYY. Data shown are representative of at least two independent experiments.

Peptide	Rat Y2a	Human Y2
NPY, human	1.3	0.74
NPY$_{2-36}$, human	2.2	1.2
NPY$_{13-36}$, human	31	2.5
NPY$_{20-36}$, porcine	93	3.6
NPY$_{26-36}$, porcine	> 830	380
NPY free acid, human	> 980	> 1000
[Leu^{31},Pro^{34}]NPY, human	. > 1000	> 130
[D-Trp^{32}]NPY, human	> 830	> 1000
PYY, porcine	0.28	0.35
PYY$_{13-36}$, porcine	1.5	28
PP, human	> 1000	> 1000
PP$_{31-36}$, human	> 10 000	> 10 000
PP, salmon	0.17	0.17
PP, bovine	> 1000	> 825
PP, rat	> 1000	> 1000

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Table 7.   Oligonucleotide probe sequences used for <u>in
situ</u> hybridization

Probe	Sequence	Location	Orientation
KS972	5'-GGC CCA TTA GGT GCA GAG GCA GAT GAG AAT CAA ACT GTA GAA GTG-3'	NH$_2$-terminus	sense
KS974	5'-CAC TTC TAC AGT TTG ATT CTC ATC TGC CTC TGC ACC TAA TGG GCC-3'	NH$_2$-terminus	antisense
KS973	5'-CGG AGG TGT CCA TGA CCT TCA AGG CTA AAA AGA ACC TGG AAG TCA-3'	COOH terminus	sense
KS975	5'-TGA CTT CCA GGT TCT TTT TAG CCT TGA AGG TCA TGG ACA CCT CCG-3'	COOH terminus	antisense

5

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Table 8.   Distribution of NPY Y2 mRNA in the rat CNS.
Positive hybridization signals are indicated by "+"
signs, no signal by "-", and a low signal by "+/-".

Region	Hybridization	Region	Hybridization
Cortex		**Hypothalamus, cont.**	
layer 2	–	tuberal	+
layer 6	–		
piriform	+	**Thalamus**	
entorhinal	–	anterior nuclei	–
cingulate	–	paraventricular n.	+
		rhomboid n.	–
Olfactory bulb	–	reuniens n.	–
Anterior olfactory n.	–	mediodorsal n.	–
		ventral nuclei	–
Basal ganglia		reticular n.	–
caudate-putamen	+/-	centrolateral n.	–
n. accumbens	–	centromedial n.	+
olfactory tubercle	+	zona incerta	–
globus pallidus	–	lateral posterior n.	–
islands of Calleja	–	lateral dorsal n.	–
		posterior n.	–
Septal area		medial geniculate n.	–
lateral septum	+	dorsal lateral gen.	–
medial septum	–	ventral lateral gen.	–
septohippocampal	–	habenula	–
diagonal band n.	–		
		Hippocampus	
Claustrum	–	CA1	–
		CA2	–
Dorsal endopiriform	–	CA3	+
		subiculum	–
Hypothalamus		presubiculum	–
anterior	–	parasubiculum	–
paraventricular	+	**Dentate gyrus**	
dorsomedial	+	granule cell layer	–
ventromedial	+	polymorph layer	–
arcuate	+		
lateral	–		
mammillary	+		

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Table 8 (continued from previous page

Region	Hybridization
Amygdala	
anterior	−
medial	+
cortical	+
amygdalohipp.	−
basomedial	+
basolateral	−
lateral	−
central	+
bed nucleus	−
Midbrain	
superior colliculus	−
inferior colliculus	−
mes. trigeminal	−
dorsal raphe	+
caudal linear raphe	+
median raphe	−
raphe magnu	−
substantia nigra	−
central gray	−
Pons/medulla	
locus coeruleus	−
subcoeruleus	−
parabrachial n.	−
facial n.	−
pontine n.	+
pontine ret. n.	−
reticulotegmental	+
A5	−
A7	−
gigantocellular	−
lateral reticular n.	−
motor trigeminal	NA
spinal trigeminal	NA
medial vestibular	−
solitarius	NA

Region	Hybridization
Pons/medulla cont.	
dorsal vagus	NA
hypoglossal	NA
Cerebellum	
granule cell layer	−
molecular layer	−
Purkinje cells	−
deep nuclei	−
Spinal cord	
dorsal horn	−
ventral horn	+
intermediolateral	−
Dorsal root ganglia	+

Abbreviations
1-9   spinal cord laminae
Arc   arcuate n. hypothalamus
BMP   posterior basomedial n. amygdala
5   CA3   field CA3 of the hippocampus
CC   central canal
Cli   caudal linear raphe n.
CM   centromedial n. thalamus
DMH   dorsomedial n. hypothalamus
10   DR   dorsal raphe n.
LSV   lateral septum, ventral
Me   medial n. amygdala
MeAV   medial n. amygdala, anteroventral division
PDTg   posterior dorsal tegmental n.
15   Pir   piriform cortex
PMD   dorsal premammillary n.
PMCo   posterior medial cortical n. amygdala
Pn   pontine n.
PVH   paraventricular n. hypothalamus
20   PVT   paraventricular n. thalamus
TC   tuber cinereum
TuO   olfactory tubercle
VMH   ventromedial n. hypothalamus

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**TABLE 9: Functional activation of the human Y2 receptor
and inhibition of cAMP accumulation.**
K_i values were derived from binding assays as described
in Table 1. Peptides were evaluated for binding affinity
and then analyzed for functional activity. Functional
data were derived from radioimmunoassay of cAMP
accumulation in stably transfected 293 cells stimulated
with 10 μM forskolin. The maximum inhibition of cAMP
accumulation relative to that produced by human NPY (E_{max})
and the concentration producing a half-maximal effect
(EC_{50}) were determined by nonlinear regression. Data
shown are representative of at least two independent
experiments.

Peptide	Binding	Function	
	K_i (nM)	EC_{50} (nM)	E_{max}
NPY, human	0.74	0.25	100 %
NPY, porcine	0.81	0.20	113%
C2-NPY, porcine	3.5	0.14	116 %
NPY_{2-36}, human	2.0	0.35	94%
NPY_{2-36}, porcine	1.2	1.2	96%
NPY_{13-36}, porcine	2.5	1.7	110 %
NPY_{16-36}, porcine	3.6	1.8	92 %
NPY_{18-36}, porcine	4.2	2.1	92 %
NPY_{20-36}, porcine	3.6	3.2	77 %
NPY_{22-36}, porcine	18	2.3	88 %
[Leu[31], Pro[34]]NPY, human	> 130	> 3000	not determined
[Leu[31], Pro[34]]NPY, porcine	> 540	> 3000	not determined
[D-Trp[32]]NPY, human	> 1000	> 3000	not determined
PYY, human	0.36	0.31	100 %

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Table 9 continued			
Peptide	Binding	Function	
	K_1 (nM)	EC_{50} (nM)	E_{max}
PYY, porcine	0.35	0.16	103 %
PYY_{3-36}, human	0.70	0.22	99 %
PYY_{13-36}, porcine	1.5	0.13	102 %
[Pro^{34}]PYY, human	> 310	> 120	not determined
PP, salmon	0.17	0.07	79 %
PYX-1	684	> 3000	not determined
PYX-2	> 1000	> 3000	not determined

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     parasympathetic innervation of the nasal mucosa.
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50
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Y and memory processing. Ann. N.Y. Acad. Sci. 611:
226-231.

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neuropeptide Y and its fragments upon basal and
electrically stimulated ion secretion in rat jejunum
mucosa. Br. J. Pharmac. 101: 247-252.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Synaptic Pharmaceutical Corporation

    (ii) TITLE OF INVENTION: NUCLEIC ACID ENCODING NEUROPEPTIDE
        Y/PEPTIDE YY (Y2) RECEPTORS AND USES THEREOF

    (iii) NUMBER OF SEQUENCES: 23

    (iv) CORRESPONDENCE ADDRESS:
        (A) ADDRESSEE: Cooper & Dunham LLP
        (B) STREET: 1185 Avenue of the Americas
        (C) CITY: New York
        (D) STATE: New York
        (E) COUNTRY: U.S.A.
        (F) ZIP: 10036

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

    (viii) ATTORNEY/AGENT INFORMATION:
        (A) NAME: White, John P.
        (B) REGISTRATION NUMBER: 28,678
        (C) REFERENCE/DOCKET NUMBER: 44742-A-PCT/JPW/MAT

    (ix) TELECOMMUNICATION INFORMATION:
        (A) TELEPHONE: 212-278-0400
        (B) TELEFAX: 212-391-0525

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1280 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 43..1185

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GACTCTTGTG CTGGTTGCAG GCCAAGTGGA CCTGTACTGA AA ATG GGT CCA ATA
    54
                                                         Met Gly Pro Ile
                                                         1

GGT GCA GAG GCT GAT GAG AAC CAG ACA GTG GAA GAA ATG AAG GTG GAA
    102
Gly Ala Glu Ala Asp Glu Asn Gln Thr Val Glu Glu Met Lys Val Glu

114

	5			10			15			20

CAA TAC GGG CCA CAA ACA ACT CCT AGA GGT GAA CTG GTC CCT GAC CCT
150
5   Gln Tyr Gly Pro Gln Thr Thr Pro Arg Gly Glu Leu Val Pro Asp Pro
               25   30   35

GAG CCA GAG CTT ATA GAT AGT ACC AAG CTG ATT GAG GTA CAA GTT GTT
198
10   Glu Pro Glu Leu Ile Asp Ser Thr Lys Leu Ile Glu Val Gln Val Val
              40   45   50

CTC ATA TTG GCC TAC TGC TCC ATC ATC TTG CTT GGG GTA ATT GGC AAC
246
15   Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu Gly Val Ile Gly Asn
              55   60   65

TCC TTG GTG ATC CAT GTG GTG ATC AAA TTC AAG AGC ATG CGC ACA GTA
294
20   Ser Leu Val Ile His Val Val Ile Lys Phe Lys Ser Met Arg Thr Val
       70   75   80

ACC AAC TTT TTC ATT GCC AAT CTG GCT GTG GCA GAT CTT TTG GTG AAC
342
25   Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala Asp Leu Leu Val Asn
    85   90   95   100

ACT CTG TGT CTA CCG TTC ACT CTT ACC TAT ACC TTA ATG GGG GAG TGG
390
30   Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr Leu Met Gly Glu Trp
             105   110   115

AAA ATG GGT CCT GTC CTG TGC CAC CTG GTG CCC TAT GCC CAG GGC CTG
438
35   Lys Met Gly Pro Val Leu Cys His Leu Val Pro Tyr Ala Gln Gly Leu
             120   125   130

GCA GTA CAA GTA TCC ACA ATC ACC TTG ACA GTA ATT GCC CTG GAC CGG
486
40   Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val Ile Ala Leu Asp Arg
           135   140   145

CAC AGG TGC ATC GTC TAC CAC CTA GAG AGC AAG ATC TCC AAG CGA ATC
534
45   His Arg Cys Ile Val Tyr His Leu Glu Ser Lys Ile Ser Lys Arg Ile
     150   155   160

AGC TTC CTG ATT ATT GGC TTG GCC TGG GGC ATC AGT GCC CTG CTG GCA
582
50   Ser Phe Leu Ile Ile Gly Leu Ala Trp Gly Ile Ser Ala Leu Leu Ala
  165   170   175   180

AGT CCC CTG GCC ATC TTC CGG GAG TAT TCG CTG ATT GAG ATC ATC CCG
630
55   Ser Pro Leu Ala Ile Phe Arg Glu Tyr Ser Leu Ile Glu Ile Ile Pro
             185   190   195

GAC TTT GAG ATT GTG GCC TGT ACT GAA AAG TGG CCT GGC GAG GAG AAG
678
60   Asp Phe Glu Ile Val Ala Cys Thr Glu Lys Trp Pro Gly Glu Glu Lys
           200   205   210

AGC ATC TAT GGC ACT GTC TAT AGT CTT TCT TCC TTG TTG ATC TTG TAT
726
65   Ser Ile Tyr Gly Thr Val Tyr Ser Leu Ser Ser Leu Leu Ile Leu Tyr
        215   220   225

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GTT TTG CCT CTG GGC ATT ATA TCA TTT TCC TAC ACT CGC ATT TGG AGT
774
Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr Thr Arg Ile Trp Ser
    230             235             240

AAA TTG AAG AAC CAT GTC AGT CCT GGA GCT GCA AAT GAC CAC TAC CAT
822
Lys Leu Lys Asn His Val Ser Pro Gly Ala Ala Asn Asp His Tyr His
245             250             255             260

CAG CGA AGG CAA AAA ACC ACC AAA ATG CTG GTG TGT GTG GTG GTG GTG
870
Gln Arg Arg Gln Lys Thr Thr Lys Met Leu Val Cys Val Val Val Val
                265             270             275

TTT GCG GTC AGC TGG CTG CCT CTC CAT GCC TTC CAG CTT GCC GTT GAC
918
Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe Gln Leu Ala Val Asp
                280             285             290

ATT GAC AGC CAG GTC CTG GAC CTG AAG GAG TAC AAA CTC ATC TTC ACA
966
Ile Asp Ser Gln Val Leu Asp Leu Lys Glu Tyr Lys Leu Ile Phe Thr
            295             300             305

GTG TTC CAC ATC ATC GCC ATG TGC TCC ACT TTT GCC AAT CCC CTT CTC
1014
Val Phe His Ile Ile Ala Met Cys Ser Thr Phe Ala Asn Pro Leu Leu
    310             315             320

TAT GGC TGG ATG AAC AGC AAC TAC AGA AAG GCT TTC CTC TCG GCC TTC
1062
Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala Phe Leu Ser Ala Phe
325             330             335             340

CGC TGT GAG CAG CGG TTG GAT GCC ATT CAC TCT GAG GTG TCC GTG ACA
1110
Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser Glu Val Ser Val Thr
                345             350             355

TTC AAG GCT AAA AAG AAC CTG GAG GTC AGA AAG AAC AGT GGC CCC AAT
1158
Phe Lys Ala Lys Lys Asn Leu Glu Val Arg Lys Asn Ser Gly Pro Asn
            360             365             370

GAC TCT TTC ACA GAG GCT ACC AAT GTC TAAGGAAGCT GTGGTGTGAA
1205
Asp Ser Phe Thr Glu Ala Thr Asn Val
            375             380

AATGTATGGA TGAATTCTGA CCAGAGCTAT GAATCTGGTT GATGGCGGCT CACAAGTGAA
1265

AACTGATTTC CCATT
1280
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(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 381 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

116

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        Met Gly Pro Ile Gly Ala Glu Ala Asp Glu Asn Gln Thr Val Glu Glu
         1           5                   10              15

   5    Met Lys Val Glu Gln Tyr Gly Pro Gln Thr Thr Pro Arg Gly Glu Leu
                     20              25              30

        Val Pro Asp Pro Glu Pro Glu Leu Ile Asp Ser Thr Lys Leu Ile Glu
                 35              40              45

  10    Val Gln Val Val Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu Gly
                 50              55              60

        Val Ile Gly Asn Ser Leu Val Ile His Val Val Ile Lys Phe Lys Ser
             65              70              75              80

  15    Met Arg Thr Val Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala Asp
                     85              90              95

        Leu Leu Val Asn Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr Leu
  20                 100             105             110

        Met Gly Glu Trp Lys Met Gly Pro Val Leu Cys His Leu Val Pro Tyr
                     115             120             125

  25    Ala Gln Gly Leu Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val Ile
                 130             135             140

        Ala Leu Asp Arg His Arg Cys Ile Val Tyr His Leu Glu Ser Lys Ile
  30        145             150             155             160

        Ser Lys Arg Ile Ser Phe Leu Ile Ile Gly Leu Ala Trp Gly Ile Ser
                     165             170             175

  35    Ala Leu Leu Ala Ser Pro Leu Ala Ile Phe Arg Glu Tyr Ser Leu Ile
                     180             185             190

        Glu Ile Ile Pro Asp Phe Glu Ile Val Ala Cys Thr Glu Lys Trp Pro
                     195             200             205

  40    Gly Glu Glu Lys Ser Ile Tyr Gly Thr Val Tyr Ser Leu Ser Ser Leu
                 210             215             220

        Leu Ile Leu Tyr Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr Thr
  45        225             230             235             240

        Arg Ile Trp Ser Lys Leu Lys Asn His Val Ser Pro Gly Ala Ala Asn
                     245             250             255

  50    Asp His Tyr His Gln Arg Arg Gln Lys Thr Thr Lys Met Leu Val Cys
                     260             265             270

        Val Val Val Val Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe Gln
                 275             280             285

  55    Leu Ala Val Asp Ile Asp Ser Gln Val Leu Asp Leu Lys Glu Tyr Lys
                 290             295             300

        Leu Ile Phe Thr Val Phe His Ile Ile Ala Met Cys Ser Thr Phe Ala
  60        305             310             315             320

        Asn Pro Leu Leu Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala Phe
                     325             330             335

  65    Leu Ser Ala Phe Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser Glu
                     340             345             350

        Val Ser Val Thr Phe Lys Ala Lys Lys Asn Leu Glu Val Arg Lys Asn
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117

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          355                  360                  365
        Ser Gly Pro Asn Asp Ser Phe Thr Glu Ala Thr Asn Val
            370                  375                  380
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(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1556 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 211..1353

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GATGAATCAG AACACAGCTA CGCAGAGGAG CTCAGCCTAA ACTAAATCAA CCCCTTTAGG
    120

ATGGTTCTCT GTTTCACTAA CTTTTTTTAA TGTCGTTTTC TGTTATAGAT TCTTGTGCTA
    180

TCTGCAGGCC AAATTGGAAC TGAGGTGAAG ATG GGC CCA TTA GGT GCA GAG GCA
    234
                                 Met Gly Pro Leu Gly Ala Glu Ala
                                  1               5

GAT GAG AAT CAA ACT GTA GAA GTG AAA GTG GAA CTC TAT GGG TCG GGG
    282
Asp Glu Asn Gln Thr Val Glu Val Lys Val Glu Leu Tyr Gly Ser Gly
        10                  15                  20

CCC ACC ACT CCT AGA GGT GAG TTG CCC CCT GAT CCA GAG CCG GAG CTC
    330
Pro Thr Thr Pro Arg Gly Glu Leu Pro Pro Asp Pro Glu Pro Glu Leu
    25                  30                  35                  40

ATA GAC AGC ACC AAA CTG GTT GAG GTG CAG GTG GTC CTT ATA CTG GCC
    378
Ile Asp Ser Thr Lys Leu Val Glu Val Gln Val Val Leu Ile Leu Ala
                    45                  50                  55

TAT TGT TCC ATC ATC TTG CTG GGC GTA GTT GGC AAC TCT CTG GTA ATC
    426
Tyr Cys Ser Ile Ile Leu Leu Gly Val Val Gly Asn Ser Leu Val Ile
                    60                  65                  70

CAT GTG GTG ATC AAA TTC AAG AGC ATG CGC ACA GTA ACC AAC TTT TTT
    474
His Val Val Ile Lys Phe Lys Ser Met Arg Thr Val Thr Asn Phe Phe
                75                  80                  85

ATT GCC AAC CTG GCT GTG GCG GAT CTT TTG GTG AAC ACC CTG TGC CTG
```

118

```
        522
        Ile Ala Asn Leu Ala Val Ala Asp Leu Leu Val Asn Thr Leu Cys Leu
            90              95              100

5       CCA TTC ACT CTT ACC TAT ACC TTG ATG GGG GAG TGG AAA ATG GGT CCA
        570
        Pro Phe Thr Leu Thr Tyr Thr Leu Met Gly Glu Trp Lys Met Gly Pro
        105             110             115             120

10      GTT TTG TGC CAT TTG GTG CCC TAT GCC CAG GGT CTG GCA GTA CAA GTG
        618
        Val Leu Cys His Leu Val Pro Tyr Ala Gln Gly Leu Ala Val Gln Val
                    125             130             135

15      TCC ACA ATA ACT TTG ACA GTC ATT GCT TTG GAC CGA CAT CGT TGC ATT
        666
        Ser Thr Ile Thr Leu Thr Val Ile Ala Leu Asp Arg His Arg Cys Ile
                    140             145             150

20      GTC TAC CAC CTG GAG AGC AAG ATC TCC AAG CAA ATC AGC TTC CTG ATT
        714
        Val Tyr His Leu Glu Ser Lys Ile Ser Lys Gln Ile Ser Phe Leu Ile
                    155             160             165

25      ATT GGC CTG GCG TGG GGT GTC AGC GCT CTG CTG GCA AGT CCC CTT GCC
        762
        Ile Gly Leu Ala Trp Gly Val Ser Ala Leu Leu Ala Ser Pro Leu Ala
            170             175             180

30      ATC TTC CGG GAG TAC TCA CTG ATT GAG ATT ATT CCT GAC TTT GAG ATT
        810
        Ile Phe Arg Glu Tyr Ser Leu Ile Glu Ile Ile Pro Asp Phe Glu Ile
        185             190             195             200

35      GTA GCC TGT ACT GAG AAA TGG CCC GGG GAG GAG AAG AGT GTG TAC GGT
        858
        Val Ala Cys Thr Glu Lys Trp Pro Gly Glu Glu Lys Ser Val Tyr Gly
                    205             210             215

40      ACA GTC TAC AGC CTT TCC ACC CTG CTA ATC CTC TAC GTT TTG CCT CTG
        906
        Thr Val Tyr Ser Leu Ser Thr Leu Leu Ile Leu Tyr Val Leu Pro Leu
                    220             225             230

45      GGC ATC ATA TCT TTC TCC TAC ACC CGG ATC TGG AGT AAG CTA AAG AAC
        954
        Gly Ile Ile Ser Phe Ser Tyr Thr Arg Ile Trp Ser Lys Leu Lys Asn
            235             240             245

50      CAC GTT AGT CCT GGA GCT GCA AGT GAC CAT TAC CAT CAG CGA AGG CAC
        1002
        His Val Ser Pro Gly Ala Ala Ser Asp His Tyr His Gln Arg Arg His
            250             255             260

55      AAA ACG ACC AAA ATG CTC GTG TGC GTG GTA GTG GTG TTT GCA GTC AGC
        1050
        Lys Thr Thr Lys Met Leu Val Cys Val Val Val Phe Ala Val Ser
        265             270             275             280

60      TGG CTG CCC CTC CAT GCC TTC CAA CTT GCT GTG GAC ATC GAC AGC CAT
        1098
        Trp Leu Pro Leu His Ala Phe Gln Leu Ala Val Asp Ile Asp Ser His
                    285             290             295

65      GTC CTG GAC CTG AAG GAG TAC AAA CTC ATC TTC ACC GTG TTC CAC ATT
        1146
        Val Leu Asp Leu Lys Glu Tyr Lys Leu Ile Phe Thr Val Phe His Ile
```

119

```
                    300                 305                 310

        ATT GCG ATG TGC TCC ACC TTC GCC AAC CCC CTT CTC TAT GGC TGG ATG
        1194
5       Ile Ala Met Cys Ser Thr Phe Ala Asn Pro Leu Leu Tyr Gly Trp Met
                315                 320                 325

        AAC AGC AAC TAC AGA AAA GCT TTC CTC TCA GCC TTC CGC TGT GAG CAG
        1242
10      Asn Ser Asn Tyr Arg Lys Ala Phe Leu Ser Ala Phe Arg Cys Glu Gln
                330                 335                 340

        AGG TTG GAT GCC ATT CAC TCG GAG GTG TCC ATG ACC TTC AAG GCT AAA
        1290
15      Arg Leu Asp Ala Ile His Ser Glu Val Ser Met Thr Phe Lys Ala Lys
        345                 350                 355                 360

        AAG AAC CTG GAA GTC AAA AAG AAC AAT GGC CTC ACT GAC TCT TTT TCA
        1338
20      Lys Asn Leu Glu Val Lys Lys Asn Asn Gly Leu Thr Asp Ser Phe Ser
                        365                 370                 375

        GAG GCC ACC AAC GTG TAAGAATGCT GTGAAAGTAC GTGGGTAAAT TGCGACCAGA
        1393
25      Glu Ala Thr Asn Val
                        380

        GTTGCCAACC TGGTTAGGGA AGGTTTTCTG GCTAGTGCAT GCCACCTCCC ATTGTATTGA
        1453
30
        CCCTAAAAGC ATCAGAGTGG AAGCCCCAGC GGTATTGTTC CTGGAAAACT GGCTGGAAGA
        1513

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35      1556
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        (2) INFORMATION FOR SEQ ID NO:4:

40   (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 381 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear

45   (ii) MOLECULE TYPE: protein

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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50       1               5                   10                  15

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                        20                  25                  30

        Pro Pro Asp Pro Glu Pro Glu Leu Ile Asp Ser Thr Lys Leu Val Glu
55                      35                  40                  45

        Val Gln Val Val Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu Gly
                50                  55                  60
60
        Val Val Gly Asn Ser Leu Val Ile His Val Val Ile Lys Phe Lys Ser
            65                  70                  75                  80

        Met Arg Thr Val Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala Asp
65                          85                  90                  95

        Leu Leu Val Asn Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr Leu
```

                                          120
                   100   105   110

          Met Gly Glu Trp Lys Met Gly Pro Val Leu Cys His Leu Val Pro Tyr
              115   120   125
5

          Ala Gln Gly Leu Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val Ile
              130   135   140
10   Ala Leu Asp Arg His Arg Cys Ile Val Tyr His Leu Glu Ser Lys Ile
          145   150   155   160

          Ser Lys Gln Ile Ser Phe Leu Ile Ile Gly Leu Ala Trp Gly Val Ser
                          165   170   175
15   Ala Leu Leu Ala Ser Pro Leu Ala Ile Phe Arg Glu Tyr Ser Leu Ile
                          180   185   190

          Glu Ile Ile Pro Asp Phe Glu Ile Val Ala Cys Thr Glu Lys Trp Pro
                  195   200   205
20
          Gly Glu Glu Lys Ser Val Tyr Gly Thr Val Tyr Ser Leu Ser Thr Leu
              210   215   220

          Leu Ile Leu Tyr Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr Thr
25   225   230   235   240

          Arg Ile Trp Ser Lys Leu Lys Asn His Val Ser Pro Gly Ala Ala Ser
                          245   250   255
30   Asp His Tyr His Gln Arg Arg His Lys Thr Thr Lys Met Leu Val Cys
                  260   265   270

          Val Val Val Val Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe Gln
              275   280   285
35
          Leu Ala Val Asp Ile Asp Ser His Val Leu Asp Leu Lys Glu Tyr Lys
              290   295   300

          Leu Ile Phe Thr Val Phe His Ile Ile Ala Met Cys Ser Thr Phe Ala
40   305   310   315   320

          Asn Pro Leu Leu Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala Phe
                          325   330   335
45   Leu Ser Ala Phe Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser Glu
                          340   345   350

          Val Ser Met Thr Phe Lys Ala Lys Lys Asn Leu Glu Val Lys Lys Asn
              355   360   365
50
          Asn Gly Leu Thr Asp Ser Phe Ser Glu Ala Thr Asn Val
              370   375   380

          (2) INFORMATION FOR SEQ ID NO:5:
55
               (i) SEQUENCE CHARACTERISTICS:
                   (A) LENGTH: 1200 base pairs
                   (B) TYPE: nucleic acid
                   (C) STRANDEDNESS: single
60   (D) TOPOLOGY: linear

              (ii) MOLECULE TYPE: genomic DNA

              (iii) HYPOTHETICAL: NO
65
              (iv) ANTI-SENSE: NO

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(ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 55..1200

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTTCTGTTAT AGATTCTTGT GCTATCTGCA GGCCAAATTG GAACTGAGGT GAAG ATG   57

                                                               Met
                                                                 1

GGC CCA TTA GGT GCA GAG GCA GAT GAG AAT CAA ACT GTA GAA GTG AAA   105
Gly Pro Leu Gly Ala Glu Ala Asp Glu Asn Gln Thr Val Glu Val Lys
              5   10   15

GTG GAA TTC TAT GGG TCG GGG CCC ACC ACT CCT AGA GGT GAG TTG CCC   153
Val Glu Phe Tyr Gly Ser Gly Pro Thr Thr Pro Arg Gly Glu Leu Pro
          20   25   30

CCT GAT CCA GAG CCG GAG CTC ATA GAC AGC ACC AAA CTG GTT GAG GTG   201
Pro Asp Pro Glu Pro Glu Leu Ile Asp Ser Thr Lys Leu Val Glu Val
      35   40   45

CAG GTG GTC CTT ATA CTG GCC TAT TGT TCC ATC ATC TTG CTG GGC GTA   249
Gln Val Val Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu Gly Val
  50   55   60   65

GTT GGC AAC TCT CTG GTA ATC CAT GTG GTG ATC AAA TTC AAG AGC ATG   297
Val Gly Asn Ser Leu Val Ile His Val Val Ile Lys Phe Lys Ser Met
                  70   75   80

CGC ACA GTA ACC AAC TTT TTT ATT GCC AAC CTG GCT GTG GCG GAT CTT   345
Arg Thr Val Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala Asp Leu
                  85   90   95

TTG GTG AAC ACC CTG TGC CTG CCA TTC ACT CTT ACC TAT ACC TTG ATG   393
Leu Val Asn Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr Leu Met
              100   105   110

GGG GAG TGG AAA ATG GGT CCA GTT TTG TGC CAT TTG GTG CCC TAT GCC   441
Gly Glu Trp Lys Met Gly Pro Val Leu Cys His Leu Val Pro Tyr Ala
          115   120   125

CAG GGT CTG GCA GTA CAA GTG TCC ACA ATA ACT TTG ACA GTC ATT GCT   489
Gln Gly Leu Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val Ile Ala
      130   135   140   145

TTG GAC CGA CAT CGT TGC ATT GTC TAC CAC CTG GAG AGC AAG ATC TCC   537
Leu Asp Arg His Arg Cys Ile Val Tyr His Leu Glu Ser Lys Ile Ser
              150   155   160

AAG CAA ATC AGC TTC CTG ATT ATT GGC CTG GCG TGG GGT GTC AGC GCT   585
Lys Gln Ile Ser Phe Leu Ile Ile Gly Leu Ala Trp Gly Val Ser Ala
              165   170   175

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CTG CTG GCA AGT CCC CTT GCC ATC TTC CGG GAG TAC TCA CTG ATT GAG
    633
Leu Leu Ala Ser Pro Leu Ala Ile Phe Arg Glu Tyr Ser Leu Ile Glu
        180             185             190

ATT ATT CCT GAC TTT GAG ATT GTA GCC TGT ACT GAG AAA TGG CCC GGG
    681
Ile Ile Pro Asp Phe Glu Ile Val Ala Cys Thr Glu Lys Trp Pro Gly
        195             200             205

GAG GAG AAG AGT GTG TAC GGT ACA GTC TAC AGC CTT TCC ACC CTG CTA
    729
Glu Glu Lys Ser Val Tyr Gly Thr Val Tyr Ser Leu Ser Thr Leu Leu
210             215             220             225

ATC CTC TAC GTT TTG CCT CTG GGC ATC ATA TCT TTC TCC TAC ACC CGG
    777
Ile Leu Tyr Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr Thr Arg
            230             235             240

ATC TGG AGT AAG CTA AAG AAC CAC GTT AGT CCT GGA GCT GCA AGT GAC
    825
Ile Trp Ser Lys Leu Lys Asn His Val Ser Pro Gly Ala Ala Ser Asp
            245             250             255

CAT TAC CAT CAG CGA AGG CAC AAA ATG ACC AAA ATG CTC GTG TGC GTG
    873
His Tyr His Gln Arg Arg His Lys Met Thr Lys Met Leu Val Cys Val
            260             265             270

GTA GTG GTG TTT GCA GTC AGC TGG CTG CCC CTC CAT GCC TTC CAA CTT
    921
Val Val Val Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe Gln Leu
        275             280             285

GCT GTG GAC ATC GAC AGC CAT GTC CTG GAC CTG AAG GAG TAC AAA CTC
    969
Ala Val Asp Ile Asp Ser His Val Leu Asp Leu Lys Glu Tyr Lys Leu
290             295             300             305

ATC TTC ACC GTG TTC CAC ATT ATT GCG ATG TGC TCC ACC TTC GCC AAC
    1017
Ile Phe Thr Val Phe His Ile Ile Ala Met Cys Ser Thr Phe Ala Asn
            310             315             320

CCC CTT CTC TAT GGC TGG ATG AAC AGC AAC TAC AGA AAA GCT TTC CTC
    1065
Pro Leu Leu Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala Phe Leu
            325             330             335

TCA GCC TTC CGC TGT GAG CAG AGG TTG GAT GCC ATT CAC TCG GAG GTG
    1113
Ser Ala Phe Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser Glu Val
            340             345             350

TCC ATG ACC TTC AAG GCT AAA AAG AAC CTG GAA GTC AAA AAG AAC AAT
    1161
Ser Met Thr Phe Lys Ala Lys Lys Asn Leu Glu Val Lys Lys Asn Asn
        355             360             365

GGC CTC ACT GAC TCT TTT TCA GAG GCC ACC AAC GTG TAA
    1200
Gly Leu Thr Asp Ser Phe Ser Glu Ala Thr Asn Val  *
370             375             380
```

(2) INFORMATION FOR SEQ ID NO:6:

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        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 382 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: protein

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

     Met Gly Pro Leu Gly Ala Glu Ala Asp Glu Asn Gln Thr Val Glu Val
      1           5                  10                  15

     Lys Val Glu Phe Tyr Gly Ser Gly Pro Thr Thr Pro Arg Gly Glu Leu
                  20                  25                  30

     Pro Pro Asp Pro Glu Pro Glu Leu Ile Asp Ser Thr Lys Leu Val Glu
              35                  40                  45

     Val Gln Val Val Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu Gly
          50                  55                  60

     Val Val Gly Asn Ser Leu Val Ile His Val Val Ile Lys Phe Lys Ser
      65                  70                  75                  80

     Met Arg Thr Val Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala Asp
                      85                  90                  95

     Leu Leu Val Asn Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr Leu
                  100                 105                 110

     Met Gly Glu Trp Lys Met Gly Pro Val Leu Cys His Leu Val Pro Tyr
                  115                 120                 125

     Ala Gln Gly Leu Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val Ile
          130                 135                 140

     Ala Leu Asp Arg His Arg Cys Ile Val Tyr His Leu Glu Ser Lys Ile
     145                 150                 155                 160

     Ser Lys Gln Ile Ser Phe Leu Ile Ile Gly Leu Ala Trp Gly Val Ser
                  165                 170                 175

     Ala Leu Leu Ala Ser Pro Leu Ala Ile Phe Arg Glu Tyr Ser Leu Ile
                  180                 185                 190

     Glu Ile Ile Pro Asp Phe Glu Ile Val Ala Cys Thr Glu Lys Trp Pro
                  195                 200                 205

     Gly Glu Glu Lys Ser Val Tyr Gly Thr Val Tyr Ser Leu Ser Thr Leu
          210                 215                 220

     Leu Ile Leu Tyr Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr Thr
     225                 230                 235                 240

     Arg Ile Trp Ser Lys Leu Lys Asn His Val Ser Pro Gly Ala Ala Ser
                  245                 250                 255

     Asp His Tyr His Gln Arg Arg His Lys Met Thr Lys Met Leu Val Cys
                  260                 265                 270

     Val Val Val Val Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe Gln
          275                 280                 285

     Leu Ala Val Asp Ile Asp Ser His Val Leu Asp Leu Lys Glu Tyr Lys
          290                 295                 300

     Leu Ile Phe Thr Val Phe His Ile Ile Ala Met Cys Ser Thr Phe Ala
```

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	305				310				315				320	

Asn Pro Leu Leu Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala Phe
325   330   335

Leu Ser Ala Phe Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser Glu
340   345   350

Val Ser Met Thr Phe Lys Ala Lys Lys Asn Leu Glu Val Lys Lys Asn
355   360   365

Asn Gly Leu Thr Asp Ser Phe Ser Glu Ala Thr Asn Val *
370   375   380

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 50 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAAGTTGTTC TCATATTGGC CTACTGCTCC ATCATCTTGC TTGGGGTAAT
    50

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 50 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATCACCACAT GGATCACCAA GGAGTTGCCA ATTACCCCAA GCAAGATGAT
    50

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 45 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTTTCATTG CCAATCTGGC TGTGGCAGAT CTTTTGGTGA ACACT
     45

(2) INFORMATION FOR SEQ ID NO:10:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 45 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: DNA (genomic)

      (iii) HYPOTHETICAL: NO

       (iv) ANTI-SENSE: NO

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGGTAAGAGT GAACGGTAGA CACAGAGTGT TCACCAAAAG ATCTG
     45

(2) INFORMATION FOR SEQ ID NO:11:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 45 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: DNA (genomic)

      (iii) HYPOTHETICAL: NO

       (iv) ANTI-SENSE: NO

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCACCTGGTG CCCTATGCCC AGGGCCTGGC AGTACAAGTA TCCAC
     45

(2) INFORMATION FOR SEQ ID NO:12:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 45 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: DNA (genomic)

      (iii) HYPOTHETICAL: NO

       (iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAGGGCAATT ACTGTCAAGG TGATTGTGGA TACTTGTACT GCCAG
    45

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 45 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AATCAGCTTC CTGATTATTG GCTTGGCCTG GGGCATCAGT GCCCT
    45

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 45 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAAGATGGCC AGGGGACTTG CCAGCAGGGC ACTGATGCCC CAGGC
    45

(2) INFORMATION FOR SEQ ID NO:15:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 46 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

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ACTGTCTATA GTCTTTCTTC CTTGTTGATC TTGTATGTTT TGCCT
   45

(2) INFORMATION FOR SEQ ID NO:16:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 45 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

   (iv) ANTI-SENSE: NO

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGTAGGAAAA TGATATAATG CCCAGAGGCA AAACATACAA GATCA
   45

(2) INFORMATION FOR SEQ ID NO:17:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 45 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

   (iv) ANTI-SENSE: NO

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTGGTGTGTG TGGTGGTGGT GTTTGCGGTC AGCTGGCTGC CTCTC
   45

(2) INFORMATION FOR SEQ ID NO:18:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 45 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

   (iv) ANTI-SENSE: NO

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TGTCAACGGC AAGCTGGAAG GCATGGAGAG GCAGCCAGCT GACCG

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45

(2) INFORMATION FOR SEQ ID NO:19:

5   (i) SEQUENCE CHARACTERISTICS:
                  (A) LENGTH: 47 base pairs
                  (B) TYPE: nucleic acid
                  (C) STRANDEDNESS: single
                  (D) TOPOLOGY: linear
10
           (ii) MOLECULE TYPE: DNA (genomic)

           (iii) HYPOTHETICAL: NO

15   (iv) ANTI-SENSE: NO

20   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

           CTCATCTTCA CAGTGTTCCA CATCATCGCC ATGTGCTCCA CTTTTGC
               47

25   (2) INFORMATION FOR SEQ ID NO:20:

           (i) SEQUENCE CHARACTERISTICS:
                  (A) LENGTH: 47 base pairs
                  (B) TYPE: nucleic acid
30   (C) STRANDEDNESS: single
                  (D) TOPOLOGY: linear

           (ii) MOLECULE TYPE: DNA (genomic)

35   (iii) HYPOTHETICAL: NO

           (iv) ANTI-SENSE: NO

40

           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

       TTCATCCAGC CATAGAGAAG GGGATTGGCA AAAGTGGAGC ACATGGC
45   47

50   (2) INFORMATION FOR SEQ ID NO:21:

           (i) SEQUENCE CHARACTERISTICS:
                  (A) LENGTH: 25 base pairs
                  (B) TYPE: nucleic acid
55   (C) STRANDEDNESS: single
                  (D) TOPOLOGY: linear

           (ii) MOLECULE TYPE: DNA (genomic)

60   (iii) HYPOTHETICAL: NO

           (iv) ANTI-SENSE: NO

65

           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGGAGTATTC GCTGATTGAG ATCAT
     25

(2) INFORMATION FOR SEQ ID NO:22:

     (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 23 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCCTTGAATG TCACGGACAC CTC
     23

(2) INFORMATION FOR SEQ ID NO:23:

     (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 45 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTGATGGTAG TGGTCATTTG CAGCTCCAGG ACTGACATGG TTCTT
     45

What is claimed is:

1.   An isolated nucleic acid molecule encoding a Y2
5   receptor.

2.   An isolated nucleic acid molecule of claim 1,
     wherein the nucleic acid molecule is a DNA molecule.

10

3.   An isolated DNA molecule of claim 2, wherein the DNA
     molecule is a cDNA molecule.

4.   An isolated DNA molecule of claim 2, wherein the DNA
15   molecule is a genomic DNA molecule.

5.   An isolated nucleic acid molecule of claim 1,
     wherein the nucleic acid molecule is a RNA molecule.

20

6.   An isolated nucleic acid molecule of claim 1 wherein
     the nucleic acid molecule encodes a human Y2
     receptor.

25   7.   An isolated nucleic acid molecule of claim 6 wherein
     the nucleic acid molecule encodes a receptor being
     characterized by an amino acid sequence in the
     transmembrane region, which amino acid sequence has
     60% homology or higher to the amino acid sequence in
30   the transmembrane region of the human Y2 receptor
     shown in Figure 11.

8.   An isolated nucleic acid molecule of claim 6 wherein
     the human Y2 receptor has substantially the same
35   amino acid sequence as shown in Figure 2.

9.   An isolated nucleic acid molecule of claim 6 wherein

the human Y2 receptor has the amino acid sequence as
shown in Figure 2.

10. An isolated nucleic acid molecule of claim 1 wherein
     the nucleic acid molecule encodes a rat Y2 receptor.

11. An isolated nucleic acid molecule of claim 10
     wherein the rat Y2 receptor has substantially the
     same amino acid sequence as shown in Figure 8.

12. An isolated nucleic acid molecule of claim 10
     wherein the rat Y2 receptor has the amino acid
     sequence shown in Figure 8.

13. An isolated nucleic acid molecule of claim 10
     wherein the rat Y2 receptor has substantially the
     same amino acid sequence as shown in Figure 9.

14. An isolated nucleic acid molecule of claim 10
     wherein the rat Y2 receptor has the amino acid
     sequence shown in Figure 9.

15. An isolated, purified Y2 receptor protein.

16. A vector comprising the nucleic acid molecule of
     claim 1.

17. A vector comprising the nucleic acid molecule of
     claim 6.

18. A vector comprising the nucleic acid molecule of
     claim 10.

19. A vector of claim 16 adapted for expression in a
     bacterial cell which comprises the regulatory
     elements necessary for expression of the nucleic
     acid in the bacterial cell operatively linked to the

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nucleic acid encoding the Y2 receptor as to permit expression thereof.

20. A vector of claim 16 adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding the Y2 receptor as to permit expression thereof.

21. A vector of claim 16 adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the Y2 receptor as to permit expression thereof.

22. A vector of claim 21 wherein the vector is a baculovirus.

23. A vector of claim 16 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the Y2 receptor as to permit expression thereof.

24. A vector of claim 17 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the Y2 receptor as to permit expression thereof.

25. A vector of claim 24 wherein the vector is a plasmid.

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26. The plasmid of claim 25 designated pcEXV-hY2 (ATCC Accession No. 75659).

27. A vector of claim 18 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the Y2 receptor as to permit expression thereof.

28. A vector of claim 27 wherein the vector is a plasmid.

29. The plasmid of claim 28 designated pcEXV-rY2a (ATCC Accession No. 97035).

30. The plasmid of claim 28 designated pcEXV-rY2b (ATCC Accession No. 97036).

31. A cell comprising the vector of either of claims 24 or 28.

32. The cell of claim 31 wherein the cell is a mammalian cell.

33. The cell of claim 32 wherein the mammalian cell is non-neuronal in origin.

34. The cell of claim 33 wherein the mammalian cell non-neuronal in origin is a COS-7 cell.

35. The cell of claim 33 wherein the mammalian cell non-neuronal in origin is a NIH-3T3 cell.

36. A NIH-3T3 cell of claim 36 designated N-hY2-5 (ATCC Accession No. CRL-11825).

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37. The cell of claim 33 wherein the mammalian cell non-
neuronal in origin is a 293 human embryonic kidney
cell.

5   38. A 293 human embryonic kidney cell of claim 37
designated 293-hY2-10 (ATCC Accession No. 11837).

39. A nucleic acid probe comprising a nucleic acid
molecule of at least 15 nucleotides capable of
10 specifically hybridizing with a unique sequence
included within the sequence of a nucleic acid
molecule encoding a Y2 receptor.

40. The nucleic acid probe of claim 39 wherein the
15 nucleic acid is DNA.

41. The nucleic acid probe of claim 39 wherein the
nucleic acid encodes a human Y2 receptor.

20 42. The nucleic acid probe of claim 39 wherein the
nucleic acid encodes a rat Y2 receptor.

43. An antisense oligonucleotide having a sequence
capable of specifically hybridizing to an mRNA
25 molecule encoding a Y2 receptor so as to prevent
translation of the mRNA molecule.

44. An antisense oligonucleotide having a sequence
capable of specifically hybridizing to the cDNA
30 molecule of claim 3.

45. An antisense oligonucleotide of either of claims 43
or 44 comprising chemical analogues of nucleotides.

35 46. An antibody directed to a Y2 receptor.

47. An antibody of claim 46, wherein the Y2 receptor is

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a human Y2 receptor.

48. An antibody of claim 46 wherein the Y2 receptor is
a rat Y2 receptor.

49. An antibody of claim 46, wherein the antibody is a
monoclonal antibody.

50. A monoclonal antibody of claim 49 directed to an
epitope of a Y2 receptor present on the surface of
a Y2 receptor expressing cell.

51. A pharmaceutical composition comprising an amount of
the oligonucleotide of claim 43 effective to
decrease activity of a Y2 receptor by passing
through a cell membrane and binding specifically
with mRNA encoding a Y2 receptor in the cell so as
to prevent its translation and a pharmaceutically
acceptable carrier capable of passing through a cell
membrane.

52. A pharmaceutical composition of claim 51, wherein
the oligonucleotide is coupled to a substance which
inactivates mRNA.

53. A pharmaceutical composition of claim 52, wherein
the substance which inactivates mRNA is a ribozyme.

54. A pharmaceutical composition of claim 51, wherein
the pharmaceutically acceptable carrier comprises a
structure which binds to a receptor on a cell
capable of being taken up by cells after binding to
the structure.

55. A pharmaceutical composition of claim 54, wherein
the structure of the pharmaceutically acceptable
carrier is capable of binding to a receptor which is

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specific for a selected cell type.

56. A pharmaceutical composition comprising an amount of
     the antibody of claim 46 effective to block binding
     of a ligand to a Y2 receptor and a pharmaceutically
     acceptable carrier.

57. A transgenic nonhuman mammal expressing nucleic acid
     encoding a Y2 receptor.

58. A transgenic nonhuman mammal comprising a homologous
     recombination knockout of the native Y2 receptor.

59. A transgenic nonhuman mammal whose genome comprises
     antisense nucleic acid complementary to nucleic acid
     encoding a Y2 receptor so placed as to be
     transcribed   into   antisense   mRNA   which   is
     complementary to mRNA encoding a Y2 receptor and
     which hybridizes to mRNA encoding a Y2 receptor
     thereby reducing its translation.

60. The transgenic nonhuman mammal of either of claims
     57 or 59, wherein the nucleic acid encoding a Y2
     receptor additionally comprises an inducible
     promoter.

61. The transgenic nonhuman mammal of either of claims
     57 or 59, wherein the nucleic acid encoding a Y2
     receptor additionally comprises tissue specific
     regulatory elements.

62. A transgenic nonhuman mammal of any of claims 57, 58
     or 59, wherein the transgenic nonhuman mammal is a
     mouse.

63. A method for determining whether a ligand can bind
     specifically to a Y2 receptor which comprises

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contacting a cell transfected with and expressing
nucleic acid encoding the Y2 receptor with the
ligand under conditions permitting binding of
ligands to such receptor, and detecting the presence
of any such ligand bound specifically to the Y2
receptor, thereby determining whether the ligand
binds specifically to a Y2 receptor.

64. A method of claim 63 wherein the Y2 receptor is a
human Y2 receptor.

65. A method of claim 63 wherein the Y2 receptor is a
rat Y2 receptor.

66. A method for determining whether a ligand can bind
specifically to a Y2 receptor, which comprises
contacting a cell transfected with and expressing
nucleic acid encoding the Y2 receptor with the
ligand under conditions permitting binding of
ligands to such receptor, and detecting the presence
of any such ligand specifically bound to the Y2
receptor, thereby determining whether the ligand
binds specifically to a Y2 receptor, wherein the Y2
receptor is characterized by an amino acid sequence
in the transmembrane region, such amino acid
sequence having 60% homology or higher to the amino
acid sequence in the transmembrane region of the Y2
receptor shown in Figure 11.

67. A method of claim 66 wherein the Y2 receptor is a
human Y2 receptor.

68. A method of claim 66 wherein the Y2 receptor is a
rat Y2 receptor.

69. A method for determining whether a ligand can bind
specifically to a Y2 receptor which comprises

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preparing a cell extract from cells transfected with
and expressing nucleic acid encoding the Y2
receptor, isolating a membrane fraction from the
cell extract, contacting the ligand with the
membrane fraction under conditions permitting
binding of ligands to such receptor, and detecting
the presence of any ligand bound to the Y2 receptor,
thereby determining whether the compound is capable
of specifically binding to a Y2 receptor.

70. A method of claim 69 wherein the Y2 receptor is a
human Y2 receptor.

71. A method of claim 69 wherein the Y2 receptor is a
rat Y2 receptor.

72. A method of any of claims 63, 64, 65, 66, 67, 68,
69, 70, or 71 wherein the ligand is not previously
known.

73. A ligand determined by the method of claim 72.

74. A method for determining whether a ligand is a Y2
receptor agonist which comprises contacting a cell
transfected with and expressing nucleic acid
encoding the Y2 receptor with the ligand under
conditions permitting the activation of a functional
Y2 receptor response from the cell, and detecting by
means of a bioassay, such as a second messenger
assay, an increase in Y2 receptor activity, thereby
determining whether the ligand is a Y2 receptor
agonist.

75. A method for determining whether a ligand is a Y2
receptor agonist which comprises preparing a cell
extract from cells transfected with and expressing
nucleic acid encoding the Y2 receptor, isolating a

membrane fraction from the cell extract, contacting
the membrane fraction of the extract with the ligand
under conditions permitting the activation of a
functional Y2 receptor response, and detecting by

5   means of a bioassay, such as a second messenger
assay, an increase in Y2 receptor activity, thereby
determining whether the ligand is a Y2 receptor
agonist.

10   76.   A method of either of claims 74 or 75 wherein the Y2
receptor is a human Y2 receptor.

77.   A method of either of claims 74 or 75 wherein the Y2
receptor is a rat Y2 receptor.

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78.   A method for determining whether a ligand is a Y2
receptor antagonist which comprises contacting a
cell transfected with and expressing nucleic acid
encoding a Y2 receptor with the ligand in the

20   presence of a known Y2 receptor agonist, such as
NPY, under conditions permitting the activation of
a functional Y2 receptor response, and detecting by
means of a bioassay, such as a second messenger
assay, a decrease in Y2 receptor activity, thereby

25   determining whether the ligand is a Y2 receptor
antagonist.

79.   A method for determining whether a ligand is a Y2
receptor antagonist which comprises preparing a cell

30   extract from cells transfected with and expressing
nucleic acid encoding the Y2 receptor, isolating a
membrane fraction from the cell extract, contacting
the membrane fraction of the extract with the ligand
in the presence of a known Y2 receptor agonist, such

35   as NPY, under conditions permitting the activation
of a functional Y2 receptor response, and detecting
by means of a bioassay, such as a second messenger ·

assay, a decrease in Y2 receptor activity, thereby determining whether the ligand is a Y2 receptor antagonist.

80. A method of either of claims 78 or 79 wherein the Y2 receptor is a human Y2 receptor.

81. A method of either of claims 78 or 79 wherein the Y2 receptor is a rat Y2 receptor.

82. A method of any of claims 74, 75, 78, or 79 wherein the second messenger assay comprises measurement of intracellular cAMP.

83. A method of any of claims 74, 75, 78, or 79 wherein the second messenger assay comprises measurement of intracellular calcium mobilization.

84. A method of any of claims 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, or 81 wherein the cell is a mammalian cell.

85. A method of claim 84 wherein the mammalian cell is nonneuronal in origin.

86. A method of claim 85, wherein the mammalian cell is nonneuronal in origin is a COS-7 cell.

87. A method of claim 85, wherein the mammalian cell nonneuronal in origin is a 293 human embryonic kidney cell.

88. The cell of claim 87 designated 293-hY2-10 (ATCC Accession No. 11837).

89. A method of claim 85, wherein the mammalian cell nonneuronal in origin is a LM(tk-) cell.

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90. A method of claim 85, wherein the mammalian cell nonneuronal in origin is a NIH-3T3 cell.

91. A cell of claim 90 designated N-hY2-5 (ATCC Accession No. CRL-11825).

92. A ligand detected by the method of any of claims 74, 75, 76, 77, 78, 79, 80, or 81.

93. A ligand of claim 92 wherein the ligand is not previously known.

94. A pharmaceutical composition comprising an amount of a Y2 receptor agonist determined by the method of either of claims 74 or 75 effective to activate a Y2 receptor and a pharmaceutically acceptable carrier.

95. A pharmaceutical composition of claim 94 wherein the Y2 receptor agonist is not previously known.

96. A pharmaceutical composition which comprises an amount of a Y2 receptor antagonist determined by the method of either of claims 78 or 79 effective to decrease activity of a Y2 receptor and a pharmaceutically acceptable carrier.

97. A pharmaceutical composition of claim 96 wherein the Y2 receptor antagonist is not previously known.

98. A method of screening drugs to identify drugs which specifically bind to a Y2 receptor on the surface of a cell which comprises contacting a cell transfected with and expressing nucleic acid encoding the Y2 receptor with a plurality of drugs under conditions permitting binding of drugs to the Y2 receptor, and determining those drugs which bind specifically to the transfected cell, thereby identifying drugs

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which bind specifically to a Y2 receptor.

99. A method of screening drugs to identify drugs which bind specifically to a Y2 receptor on the surface of a cell which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding the Y2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs under conditions permitting binding of drugs to the Y2 receptor, and determining those drugs which bind specifically to the transfected cell, thereby identifying drugs which bind specifically to a Y2 receptor.

100. A method of either of claims 98 or 99 wherein the Y2 receptor is a human Y2 receptor.

101. A method of either of claims 98 or 99 wherein the Y2 receptor is a rat Y2 receptor.

102. A method of screening drugs to identify drugs which act as agonists of a Y2 receptor which comprises contacting a cell transfected with and expressing nucleic acid encoding the Y2 receptor with a plurality of drugs under conditions permitting the activation of a functional Y2 receptor response, and determining those drugs which activate such receptor using a bioassay, such as a second messenger assay, thereby identifying drugs which act as agonists of a Y2 receptor.

103. A method of screening drugs to identify drugs which act as agonists of a Y2 receptor which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding the Y2 receptor, isolating a membrane fraction from the

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cell extract, contacting the membrane fraction with
a plurality of drugs under conditions permitting the
activation of a functional Y2 receptor response, and
determining those drugs which activate such receptor
using a bioassay, such as a second messenger assay,
thereby identifying drugs which act as agonists of
a Y2 receptor.

104. A method of either of claims 102 or 103 wherein the
      Y2 receptor is a human Y2 receptor.

105. A method of either of claims 102 or 103 wherein the
      Y2 receptor is a rat Y2 receptor.

106. A method of screening drugs to identify drugs which
      act as antagonists of Y2 receptors which comprises
      contacting a cell transfected with and expressing
      nucleic acid encoding a Y2 receptor with a plurality
      of drugs in the presence of a known Y2 receptor
      agonist such as NPY under conditions permitting the
      activation of a functional Y2 receptor response, and
      determining those drugs which inhibit the activation
      of the receptor using a bioassay, such as a second
      messenger assay, thereby identifying drugs which act
      as antagonists of Y2 receptors.

107. A method of screening drugs to identify drugs which
      act as antagonists of Y2 receptors which comprises
      preparing a cell extract from cells transfected with
      and   expressing   nucleic   acid   encoding   the   Y2
      receptor, isolating a membrane fraction from the
      cell extract, contacting the membrane fraction with
      a plurality of drugs in the presence of a known Y2
      receptor   agonist   such   as   NPY   under   conditions
      permitting the activation of a functional Y2
      receptor response, and determining those drugs which
      inhibit the activation of the receptor using a

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bioassay, such as a second messenger assay, thereby identifying drugs which act as antagonists of Y2 receptors.

108. A method of either of claims 106 or 107 wherein the Y2 receptor is a human Y2 receptor.

109. A method of either of claims 106 or 107 wherein the Y2 receptor is a rat Y2 receptor.

110. A method of any of claims 102, 103, 106 or 107 wherein the second messenger assay comprises measurement of intracellular cAMP.

111. A method of any of claims 102, 103, 106, or 107 wherein the second messenger assay comprises measurement of intracellular calcium mobilization.

112. A method of any of claims 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, or 109 wherein the cell is a mammalian cell.

113. A method of claim 112 wherein the mammalian cell is nonneuronal in origin.

114. The method of claim 113 wherein the mammalian cell nonneuronal in origin is a Cos-7 cell.

115. The method of claim 113 wherein the mammalian cell nonneuronal in origin is a 293 human embryonic kidney cell.

116. The cell of claim 115 designated 293-hY2-10 (ATCC Accession No. 11837).

117. The method of claim 113 wherein the mammalian cell nonneuronal in origin is a LM(tk-) cell.

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118. The method of claim 113 wherein the mammalian cell nonneuronal in origin is a NIH-3T3 cell.

119. The cell of claim 118 designated N-hY2-5 (ATCC Accession No. CRL-11825).

120. A pharmaceutical composition comprising an effective amount of a drug identified by the method of either of claims 102 or 103 and a pharmaceutically acceptable carrier.

121. A pharmaceutical composition comprising an effective amount of a drug identified by the method of either of claims 106 or 107 and a pharmaceutically acceptable carrier.

122. A method of detecting expression of a Y2 receptor by a cell by detecting the presence of mRNA coding for a Y2 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of claim 39 under hybridizing conditions, and detecting the presence of mRNA hybridized to the probe, thereby detecting the expression of Y2 receptor by the cell.

123. A method of treating an abnormality in a subject, wherein the abnormality is alleviated by activation of a Y2 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of either of claims 94 or 120, thereby treating the abnormality.

124. A method of treating an abnormality in a subject, wherein the abnormality is alleviated by activation of a Y2 receptor which comprises administering to a subject an effective amount of Y2 receptor agonist determined by any of claims 74, 75, 102, or 103,

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thereby treating the abnormality.

125. A method of treating an abnormality in a subject, wherein the abnormality is alleviated by decreasing the activity of a Y2 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of either of claims 96 or 121, thereby treating the abnormality.

126. A method of treating an abnormality in a subject, wherein the abnormality is alleviated by decreasing the activity of a Y2 receptor which comprises administering to the subject an effective amount of a Y2 receptor antagonist determined by the methods of any of claims 78, 79, 106, or 107, thereby treating the abnormality.

127. The method of either of claims 125 or 126 wherein the abnormality is a cognitive disorder.

128. The method of either of claims 125 or 126 wherein the abnormality is a gastrointestinal disorder.

129. The method of either of claims 125 or 126 wherein the abnormality is sleeping disorder.

130. The method of either of claims 125 or 126 wherein the abnormality is epilepsy.

131. The method of either claims 125 or 126 wherein the abnormality is hypertension.

132. The method of either of claims 123 or 124 wherein the abnormality is memory loss.

133. The method of either of claims 123 or 124 wherein the abnormality is diarrhea.

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134. The method of either of claims 123 or 124 wherein the abnormality is nasal congestion.

135. The method of either of claims 123 or 124 wherein the abnormality is pain.

136. A method of treating an abnormality in a subject, wherein the abnormality alleviated by decreasing the activity of a Y2 receptor which comprises administering to the subject an amount of the pharmaceutical composition of claim 56 effective to block binding of ligands to the Y2 receptor, thereby treating the abnormality.

137. A method of treating an abnormality in a subject, wherein the abnormality is alleviated by decreasing the activity of a Y2 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition of claim 51, thereby treating the abnormality.

138. The method of either of claims 136 or 137 wherein the abnormality is a cognitive disorder.

139. The method of either of claims 136 or 137 wherein the abnormality is a gastrointestinal disorder.

140. The method of either of claims 136 or 137 wherein the abnormality is epilepsy.

141. The method of either of claims 136 or 137 wherein the abnormality is hypertension.

142. The method of either of claims 136 or 137 wherein the abnormality is sleeping disorder.

143. A method of detecting the presence of a Y2 receptor

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on the surface of a cell which comprises contacting the cell with the antibody of claim 46 under conditions permitting binding of the antibody to the receptor, and detecting the presence of the antibody bound to the cell, thereby detecting the presence of a Y2 receptor on the surface of the cell.

144. A method of determining the physiological effects of expressing varying levels of Y2 receptors which comprises producing a transgenic nonhuman mammal of claim 55 whose levels of human Y2 receptor expression are varied by use of an inducible promoter which regulates Y2 receptor expression.

145. A method of determining the physiological effects of expressing varying levels of Y2 receptors which comprises producing a panel of transgenic nonhuman mammals of claim 55 each expressing a different amount of Y2 receptor.

146. A method for identifying a Y2 receptor antagonist capable of alleviating an abnormality in a subject, wherein the abnormality is alleviated by decreasing the activity of a Y2 receptor which comprises administering the antagonist to a transgenic nonhuman mammal of any of claims 57, 58, or 59 and determining whether the antagonist alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of activity of a Y2 receptor, thereby identifying a Y2 antagonist.

147. An antagonist identified by the method of claim 146.

148. A pharmaceutical composition comprising an effective amount of an antagonist identified by the method of claim 146 and a pharmaceutically acceptable carrier.

149. A method for treating an abnormality in a subject
      wherein the abnormality is alleviated by decreasing
      the activity of a Y2 receptor which comprises
      administering to the subject an effective amount of
      the pharmaceutical composition of claim 148, thereby
      treating the abnormality.

150. A method for identifying a Y2 receptor agonist
      capable of alleviating an abnormality wherein the
      abnormality is alleviated by activation of a Y2
      receptor which comprises administering the agonist
      to the transgenic nonhuman mammal of any of claims
      57, 58, or 59 and determining whether the agonist
      alleviates the physical and behavioral abnormalities
      displayed by the transgenic nonhuman mammal, thereby
      identifying a Y4 receptor agonist.

151. An agonist identified by the method of claim 150.

152. A pharmaceutical composition comprising an effective
      amount of an agonist identified by the method of
      claim 150 and a pharmaceutically acceptable carrier.

153. A method for treating an abnormality in a subject
      wherein the abnormality is alleviated by activation
      of a Y2 receptor which comprises administering to
      the   subject   an   effective   amount   of   the
      pharmaceutical composition of claim 152, thereby
      treating the abnormality.

154. A method for diagnosing a predisposition to a
      disorder associated with the activity of a specific
      Y2 receptor allele which comprises:

      a.   obtaining nucleic acid of subjects

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suffering from the disorder;

b.   performing a restriction digest of the nucleic acid with a panel of restriction enzymes;

c.   electrophoretically separating the resulting nucleic acid fragments on a sizing gel;

d.   contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to nucleic acid encoding a Y2 receptor and labelled with a detectable marker;

e.   detecting labelled bands which have hybridized to the nucleic acid encoding a Y2 receptor labelled with a detectable marker to create a unique band pattern specific to the nucleic acid of subjects suffering from the disorder;

f.   preparing nucleic acid obtained for diagnosis by steps a-e; and

g.   comparing the unique band pattern specific to the nucleic acid of subjects suffering from the disorder from step e and the nucleic acid obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

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155. The method of claim 154 wherein a disorder associated with the expression of a specific Y2 receptor allele is diagnosed.

156. A method of preparing the isolated, purified Y2 receptor of claim 15 which comprises:

a.   constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid in the cell operatively linked to the nucleic acid encoding a Y2 receptor as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;

b.   inserting the vector of step (a) in a suitable host cell;

c.   incubating the cells of step (b) under conditions allowing the expression of a Y2 receptor;

d.   recovering the receptor so produced;

e.   purifying the receptor so recovered.

FIGURE 1

```
   1  GACTCTTGTGCTGGTTGCAGGCCAAGTGGACCTGTACTGAAAATGGTCCAATAGGTGCA     60
  61  GAGGCTGATGAGAACCAGAGTGGAAGAAATGAAGGTGGAACAATACGGGCCACAAACA    120
 121  ACTCCTAGAGGTGAACTGGTCCCTGACCCTGAGCCAGAGCTTATAGATAGTACCAAGCTG   180
 181  ATTGAGGTACAAGTTGTTCTCATATTGGCCTACTGCTCCATCATCTTGCTTGGGGTAATT   240
 241  GGCAACTCCTTGGTATCCATGTGGTGATCAAATTCAAGAGCATGCGCACAGTAACCAAC    300
 301  TTTTCATTGCCAATCTGGCTGTGTGGCAGATCTTTTGGTGAACACTCTGTGTCTACCGTTC  360
 361  ACTCTTACCTATACCTTAATGGGGAGTGGAAAATGGGGTCCTGCCACCTGGTG          420
 421  CCCTATGCCCCAGGGCCTGGCAGTACAAGTATCCACAACCTTGACACAGTAATTGCCCTG   480
 481  GACCGGCACAGTGCATCGTTCTACCACCTAGAGAGCAAGATCTCCAAGCGAATCAGCTTC   540
 541  CTGATTATTGGCTTGGCCTTGATTGAGATCATCCCGGACTTTGAGATTGTGGCCTGTACTGAAAAG  600
 601  CGGGAGTATTCGCTGATTGAGATCATCCCGGACATCTATAGTCTTTCTTGTTGATC       660
 661  TGGCCTGGCGAGGAGAGAAGCATCTATGGCCATTATATCATTTCCTACACTCCGCATTTGGAGTAAATTG  720
 721  TTGTATGTTTTGCCTCTGGGCATTATATCATTTCCTACACTCCGCATTTGGAGTAAATTG   780
 781  AAGAACCATGTCAGTCTCGGAGCTGCAAATGACCACTACCATCAGCGAAGGCAAAAACC    840
 841  ACCAAATGCTGGTGTGTCCGTTGTGTGTTGCGTGGTGGTCAGCTGGCCTCTCCATGCC    900
 901  TTCCAGCTTGCCGTTGACATTGACAGCCAGTCCTGAAGGAGTACAAACTCATC          960
 961  TTCACAGTGTTCCACATCATCGCCATGTGCTCCCACTTTGCCAATCCCTGTGAGCAGCGGTTG  1020
1021  TGGATGAACACAGCAACTACAGAAAGGCTTTCCCTCTCCGCCTTCAAGGCTAAAAAGAACC 1080
1081  GATGCCATTCACTCTGAGGTGTCCGTGACATTCAAGAGGCTACCAATGTCTAAGGAGTCAGA 1140
1141  AAGAACAGTGCCCCCAATGACTCTTTCACAGAGGCTACCAATGTCTAAGGAAGCTGTGGT    1200
1201  GTGAAAATGTATGGGATGAATTCTGACCAGAGCTATGAATCTGGTTGATGGCGGCTCACAA  1260
1261  GTGAAAACTGATTTCCCATT    1280
```

FIGURE 2

```
        6   26  46  66  86  106 126 146 166 186 206 226 246 266 286 306 326 346 366 381

        A   T   L   V   N   I   F   L   F   F   K   I   L   T   A   I   G   L   R
        G   Q   K   L   V   P   A   S   I   E   L   K   H   L   Y   R   V
        I   P   P   T   H   V   G   L   H   I   A   T   L   S   Q   L   K   L   Q   E
        P   G   S   C   T   L   C   V   R   L   C   S   W   R   P   Y   L   E   L
        G   Y   D   L   R   L   L   T   K   P   A   S   I   R   L   E   P   C   N   *
        M   Q   I   V   M   I   T   L   S   S   V   L   R   Q   W   K   N   R   K   V
        E   L   P   S   I   N   T   I   A   I   S   T   H   S   L   A   F   K   N
        V   E   G   K   S   V   H   K   L   E   Y   Y   Y   A   V   D   F   A   A   T
        K   P   M   F   C   L   T   S   L   F   V   S   H   A   L   T   S   K   A
        E   E   K   Y   L   S   E   A   D   T   F   D   F   V   S   L   F   E   A
        E   P   W   I   A   D   V   L   S   P   G   S   N   V   Q   C   F   T   T
        E   D   E   V   L   A   Q   H   I   Y   I   A   V   S   M   A   V   F
        V   P   G   V   I   V   V   Y   G   I   I   A   V   D   A   K   V   S
        T   V   M   H   L   A   A   V   W   E   S   G   V   I   R   V   D
        Q   L   L   I   V   L   L   I   A   I   K   L   P   C   D   I   Y   E   N
        N   E   T   V   V   N   G   C   L   L   E   P   S   V   V   H   N   S   P
        E   G   Y   L   Q   A   Q   R   G   S   E   L   V   L   A   F   S   H   G
        D   R   T   S   V   I   A   H   I   Y   G   V   H   M   L   V   N   I   S
        A   D   P   L   N   E   F   Y   R   I   E   P   Y   N   K   Q   T   M   A   N
        E   T   L   G   I   F   P   D   L   R   W   L   K   T   F   F   W   D   K

        7   27  47  67  87  107 127 147 167 187 207 227 247 267 287 307 327 347 367
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FIGURE 3A
FIGURE 3B
FIGURE 3C
FIGURE 3D

FIGURE 3A

```
1002 ATGGGTCCAATAGGTGCAGAGGCTGATGAGAACCAGACAGTGGAAGAAAT 1051
                        |          ||
197  ..........................ATGAATTCAACATTATTTTC 216

1052 GAAGGTGGAACAATACGGGCCACAAACAACTCCTAGAGGTGAACTGGTCC 1101
        |||| |||                              |  |
217  CCAGGTTGAAAATCATTCAGTCCACTCTAATTTCTCAGAGAAGAATGCCCC 266

1102 CTGACCCCTGAGCCAGAGCTTATAGATAGTACCAAGCTGATTGAGGTACAA 1151
          |           |||     ||          |
267  AGCTTCTGGCTTTTGAAAATGATGATTGTCATCTGCCCCTTGGCCATGATA 316

1152 GTTGTTCTCATATTGGCCTACTGCTCCATCATCTTGCTTGGGGTAATTGG 1201
        ||                | || ||  |  ||||| |||
317  TTTACCTTAGCTCTTGCTTATGGAGCTGTGATCATTCTTGGTGTCTCTGG 366

1202 CAACTCCTTGGTGATCCATGTGGTGATCAAATTCAAAGAGCATGCGCACAG 1251
          |||   |||||                |||     ||
367  AAACCTGGCCTTGATCATAATCATCTTGAAACAAAGGAGATGAGAAATG 416

1252 TAACCAACTTTTTCATTGCCAATCTGGCTGTGGCAGATCTTTTGGTGAAC 1301
          |||||||  | ||||||          ||       ||||
417  TTACCAACATCCTGATTGTGAACCTTTCCTTCTCAGACTTGCTTGTTGCC 466
```

FIGURE 3B

```
1302  ACTCTGTGTCTACCGTTCACTCTTACCTATACCTTAATGGGGGAGTGGAA  1351
            ||||||  || ||| ||| || |||  ||  ||||||   |    |||
 467  ATCATGTGTCTCCCCTTTACATTTGTCTACACATTAATGGACCACTGGGT   516

1352  AATGGGTCCTGTCCTGTGCCACCTGGTGCCCCTATGCCCCAGGGCCTGGCAG  1401
         |   ||||                  || | || ||  ||      ||
 517  CTTTGGTGAGGCGATGTGTAAGTTGAATCCTTTGTGCAAATGTGTTTCAA   566

1402  TACAAGTATCCACAATCACCTTGACACAGTAATTGCCCCTGGACCGGCACAGG  1451
         |   || || ||||  ||  ||  |  |||| |||||||  ||   ||
 567  TCACTGTGTCCATTTTCTCTGGTTCTCTGCTGTGGAACGACATCAG   616

1452  TGCATCGTCTACCACCTAGAGAGCAAGATCTCCAAGCGAATCAGCTTCCT  1501
        ||  || ||| ||| | |  ||      |  ||     ||   |  |
 617  CTGATAATCAACCCCTCGAGGGTGGAGACCAAATAATAGACATGCTTATGT  666

1502  GATTATTGGCTTGGCCTGGGGCATCAGTGCCCTGCTGGCAAGTCCCCCTGG  1551
         ||||   || |  |  ||| |           |||    |||
 667  AGGTATTGCTGTGATTTGGGTCCTTGCTG..TGGCTTCTTCTTTGCCTTT  714

1552  CCATCTTCCGGGAGTATTCGCTGATTGAGATCATCCCGGACTTTGAGATT  1601
        ||  ||  ||||||    ||||  ||||    ||   ||
 715  CCTGATCTACCAAGTAATGACTGA.TGAGCCGTTCCAAAATGTAACACTT  763
```

FIGURE 3C

```
1602  GTGGCCTGTACTGAAAAGTGGCCTGGCGAGGAGAA..........GAGCAT  1642
              ! !  !  !! !!                            ! !
 764  GATGCGTACAAAGACAAATACGTGTGCTTTGATCAATTCCATCGGACTC    813

1643  CTATGGCACTGTCTATAGTCTTTCTTCCTTGTTGATCTTGTATGTTTGC   1692
              !!!!       !     !!!! !!!!  !  !
 814  TCATAGGTGTGTCTTATACCACTCTCCTCCTTGGCTGCAGTATTTTGGTC   863

1693  CTCTGGGCATTATATCATTTTCCTACACTCGCATTT..........GGAGTA  1734
       !! !!!!!!   !!! !!!!                          ! !
 864  CACTTTGTTTTATATTTATTTGCTACTTCAAGATATATATACGCCTAAAA   913

1735  AATTGAAGAACCATGTCAGTCCTGGAGCTGCAAATGACCACTACCATCAG  1784
       !  ! !!!!  !! !                        !  !!!
 914  AGGAGAAACAACATGATGGACAAGATGAGAGAGAACAATAAGTACAGGTCCAG  963

1785  CGAAGGCAAAA.AACCACCAAAAATGCTGGTGGTGGTGGTGGTGGTGTTG  1833
       !!! !!!!!!  !  !  !   !    !     !!!! !!!!
 964  TGAAACCAAAAGAATCAATATCATGCTGCTCTCCATTGTGGTAGCATTTG   1013

1834  CGGTCAGCTGGCCCTCTGCCCTTCCATGCCTTCCAGCTTGCCGTTGACATTGAC  1883
       !!! !!!!!! !!!!!       !              !  !!!
1014  CAGTCTGCTGGCTCCCCTCTTACCATCTTTAACACTGTGTTTGATTGGAAT  1063
```

FIGURE 3D

```
1884  AGCCAGGTCCTGGACCTGAAGGAGTACAAACTCATCTTCACAGTGTTCCA  1933
                   - ||  ||     ||       - |  |||
1064  CATCAGATCATTGCTACCTGCAACCACAATCTGTTATTCCTGCTCTGCCA  1113

1934  CATCATCGCCATGTGCTCCACTTTGCCAATCCCCTTCTCTATGGCTGGA  1983
      | ||  ||  ||| |||||||  |     |   |||  -
1114  CCTCACAGCAATGATATCCACTTGTGTCAACCCCATATTTTATGGGTTCC  1163

1984  TGAACAGCAACTACAGAAAGGCTTTCCTCTCGGCCTTCCGC....TGTGAG  2030
      |||||| ||| ||||         -  | -  ||        |||||
1164  TGAACAAAACTTCCAGAGAGACTTGCAGTTCTTCTTCAACTTTTGTGAT  1213

2031  CAGCGGTTGGATGCCATTCACTCTGAGGTGTCCGTGACATTCAAG......  2075
           |||| |   -  | ||| |   - |  ||  |   - ||
1214  TTCCGGTCTCGGGATGATGATTATGAAACAATAGCCATGTCCACGATGCA  1263

2076  .......GCTAAAAAGAACCTGGAGGTCAGAAAGAACAGTGGC.CCCA  2115
               ||||       |  - | ||| ||   | ||
1264  CACAGATGTTTCCAAAACTTCTTTGAAGCAAGCCCAGTCGCGCATTTA  1313

2116  ATGACTCTTTCACAGAGGCTACCAATGTCTAA.......  2147
      - |   ||    |||||||     ||   |||
1314  AAAAAATCAACAACAATGATGATAATGAAAAAATCTGA  1351
```

FIGURE 4A

FIGURE 4A
FIGURE 4B

```
  1  MGPIGAEADENQTVEEMKVEQYGPQTTPRGELVPDPEPELIDSTKLIEVQ  50
     |... . ||:.|.       .:|:...:.:         |:   :.:
  1  MNSTLFSQVENHSVH.....SNFSEKNAQLLAFEND......DCHLPLAMI  40

 51  VVLILAYCSILLGVIGNSLVIHVVIKFKSMRTVTNFFIANLAVADLLVN  100
     ..| |||...:|:||| ||   :| |.||:||:.||.|....||||.
 41  FTLALAYGAVIILGVSGNLALIIILKQKEMRNVTNLLIVNLSFSDLLVA   90

101  TLCLPFTLTYTLMGEWKMGPVLCHLVPYAQGLAVQVSTITLTVIALDRHR  150
     .:||||||.||||:.|  :|.:.:|  |:.:.: ||.:.:.::|.::|.
 91  IMCLPFTFVYTLMDHWVFGEAMCKLNPFVQCVSITVSIFSLVLIAVERHQ  140

151  CIVYHLESKISKRISFLIIGLAWGISALLASPLAIFREYSLIEIIPDFEI  200
     |:. .::  ..|.:.  |::  |.:.:  |. |: |:  .:..::
141  LIINPRGWRPNNRHAYVGIAVIWVLAVASSLPFLIYQVMT.DEPFQNVTL  189
```

FIGURE 4B

```
201 VACTEKW.......PGEEKSIYGTVYSLSSLLILYVLPLGIISFSYTRIW 243
        |::.:|:          .::.|  |.|:.       |::  |.  |.:|  ::|  :|:
190 DAYKDKYVCFDQFPSDSHRLSYTTLL.....LVLQYFGPLCFIFICYFKIY 235

244 SKLK..NHVSPGAANDHYHQRRQKTTK.MLVCVVVVFAVSWLPLHAFQLA 290
       :||  |::.     ::.|:  .  |..  ||:::||.||:|||   |.
236 IRLKRRNNMMDKMRDNKYRSSETKRINIMLLSIVVAFAVCWLPLTIFNTV 285

291 VDIDSQVLDLKEYKLIFTVFHIIAMCSTFANPLLYGWMNSNYRKAF..... 336
       .|.|:|.  .:.|:|  |:.|:.||  .||::||:.|:.:.:
286 FDWNHQIIATCNHNLLFLLCHLTAMISTCVNPIFYGFLNKNFQRDLQFFF 335

337 .LSAFRCEQR.......LDAIHSEVSVT.FKAKKNLEVRK..NSGPNDSFT 376
       ::.:||: :       .:.:.:|||   .:. .:..:|   |.:.|:.:.
336 NFCDFRSRDDDYETIAMSTMHTDVSKTSLKQASPVAFKKINNNDDNEKI * 385

377 EATNV* 381
```

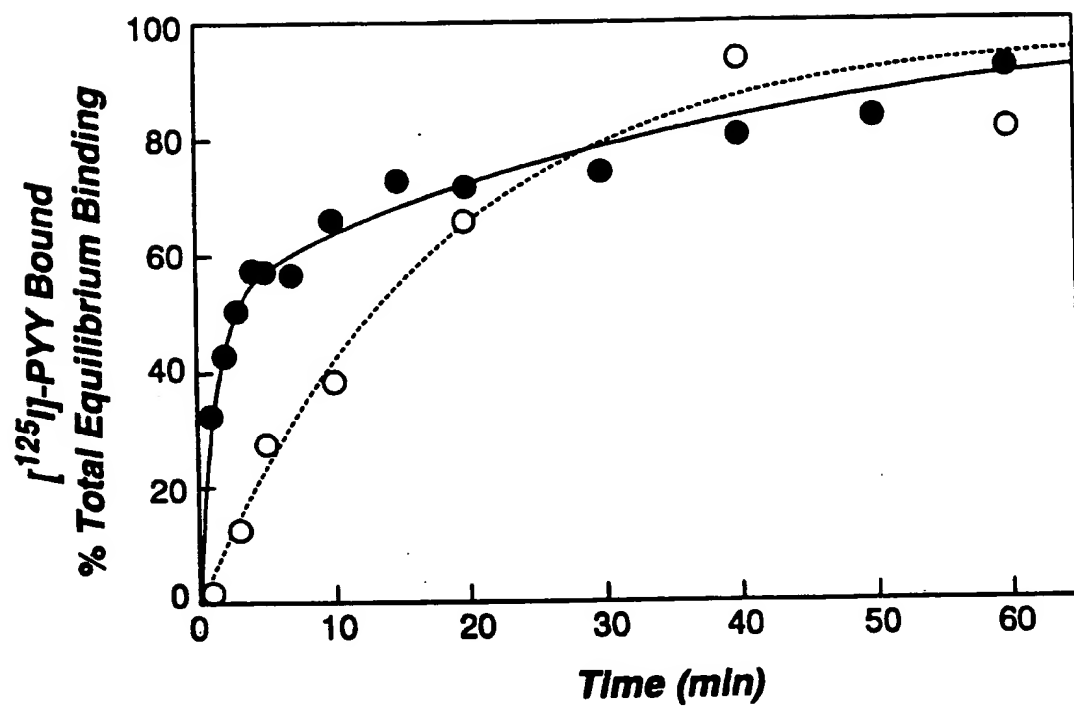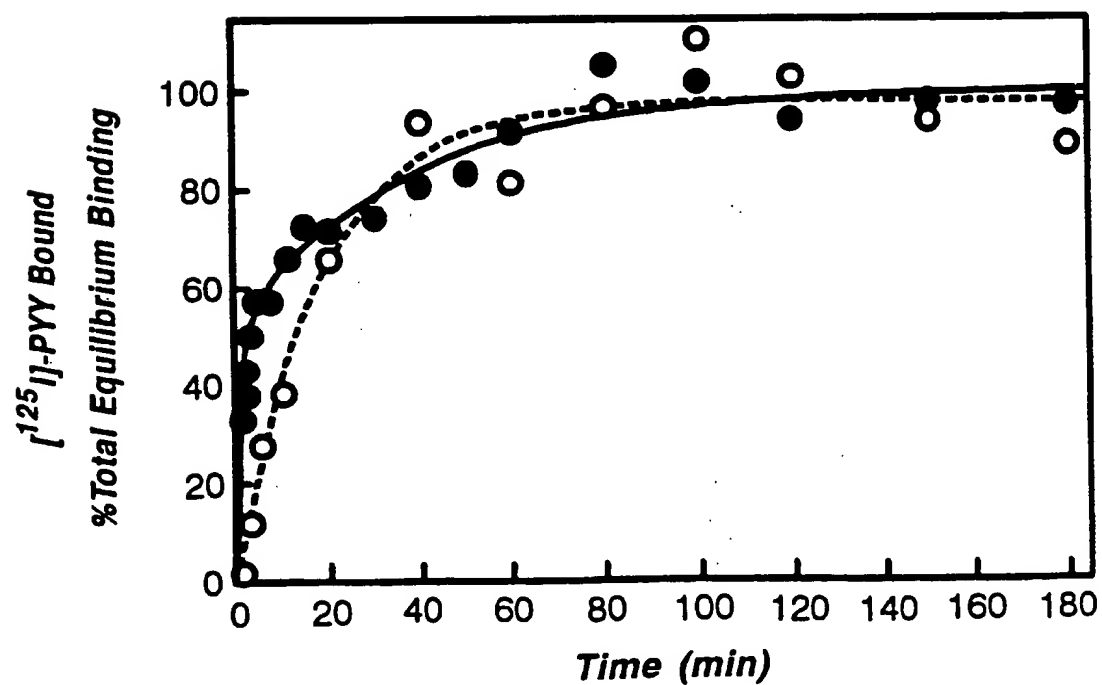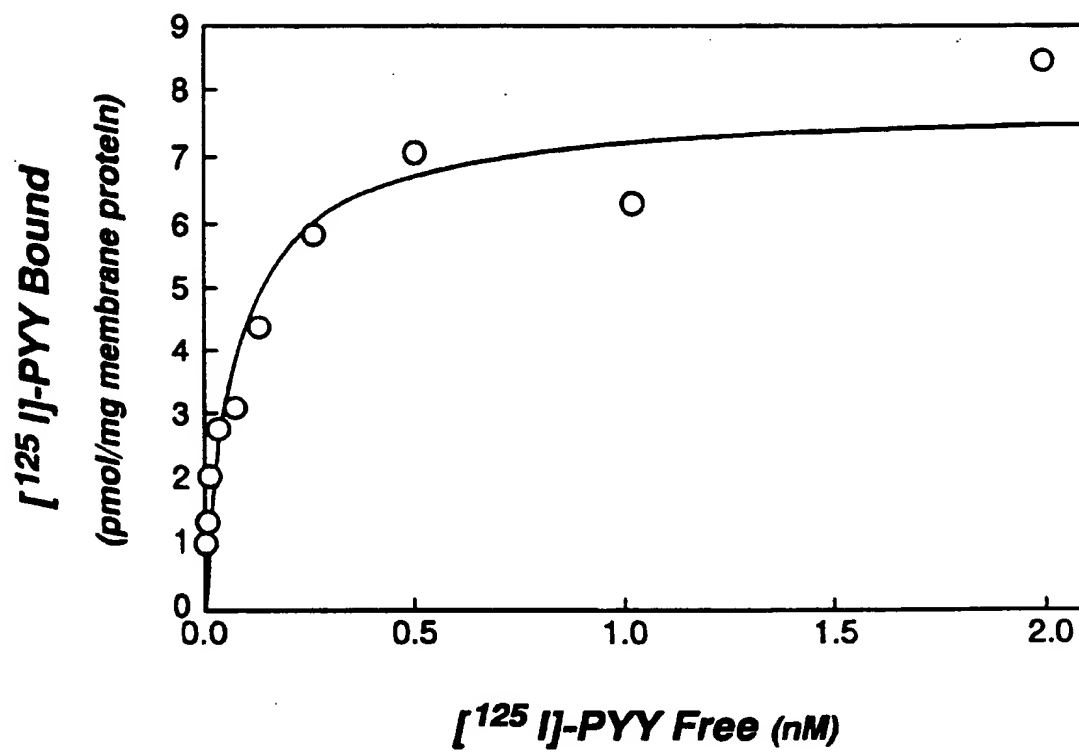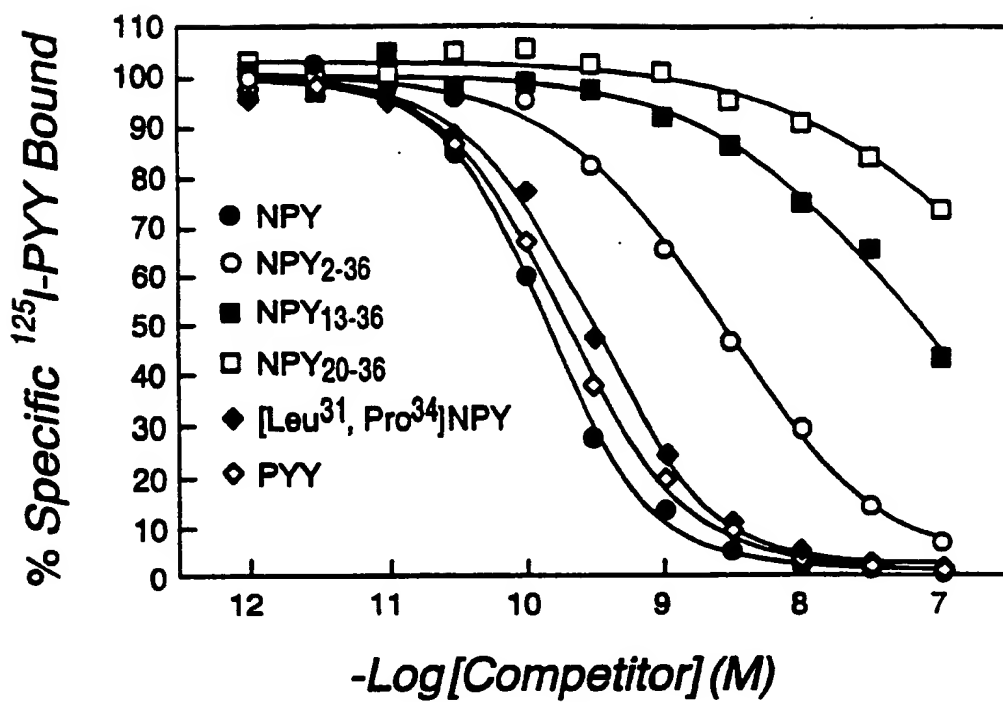## FIGURE 5A

FIGURE 5B

FIGURE 6

FIGURE 7A

FIGURE 7B
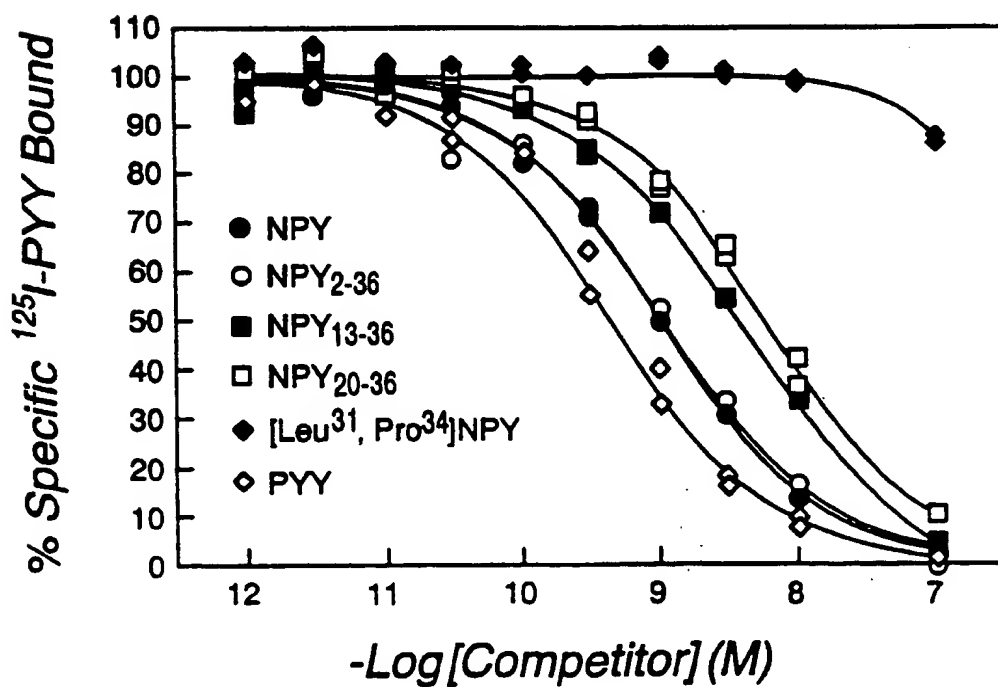
12/35

FIGURE 8A

FIGURE 8A
FIGURE 8B
FIGURE 8C
FIGURE 8D
FIGURE 8E

```
                                                 -160
               -180
       -200
GTTGTTAACAGACTCGTGTGTAAAGGATTTGCTTTTATGGAGCTTTTATGAGATCTGTGGTGT

                                                 -100
               -120
       -140
GATGAATCAGAGAACACAGCTACGCGCAGAGGAGCTCAGCCTAAACTAAATCAACCCCTTTAGG

                                                 -40
               -60
       -80
ATGGTTCTCTGTTTCACTAACTTTTTTTAATGTCGTTTTTCTGTTTATAGATTCTTGTGCTA

                                                 20
               1
       -20
TCTGCAGGCCAAATTGGAACTGAGGTGAAGATGGGCCCATTAGGTGCAGAGGCAGAGATGAG
                                M  G  P  L  G  A  E  A  D  E

                                                 80
               60
       40
AATCAAACTGTAGAAGTGAAAGTGGAACTCTATGGGTCGGGGCCCACCACTCCTAGAGGT
N  Q  T  V  E  V  K  V  E  L  Y  G  G  S  G  P  T  T  P  R  G
```

FIGURE 8B

```
        100        120        140
GAGTTGCCCCCTGATCCAGAGAGCCGGAGCTCATAGACAGCACCAAACTGGTTGAGGTGCAG
 E  L  P  P  D  P  E  P  E  L  I  D  S  T  K  L  V  E  V  Q

        160        180        200
GTGGTCCTTATACTGGCCTATTGTTCCATCATCTTGCTGGGCGTAGTTGGCAACTCTCTG
 V  V  L  I  L  A  Y  C  S  I  I  L  L  G  V  V  G  N  S  L

        220        240        260
GTAATCCATGTGGTGATCAAATTCAAGAGCATGCGCACAGTAACCAACTTTTTTATTGCC
 V  I  H  V  V  I  K  F  K  S  M  R  T  V  T  N  F  F  I  A

        280        300        320
AACCTGGCTGTGGCGGATCTTTTGGTGAACACCCTGTGCCTGCCATTCACTCTTACCTAT
 N  L  A  V  A  D  L  L  V  N  T  L  C  L  P  F  T  L  T  Y

        340        360        380
ACCTTGATGGGGGAGTGGAAAATGGGTCCAGTTTTGTGCCATTTGGTGCCCTATGCCCAG
 T  L  M  G  E  W  K  M  G  P  V  L  C  H  L  V  P  Y  A  Q
```

FIGURE 8C

```
                400           420           440
GGTCTGGCAGTACAAGTGTCCACAATAACTTTGACAGTCATTGCTTTGGACCGACATCGT
 G  L  A  V  Q  V  S  T  I  T  L  T  V  I  A  L  D  R  H  R

                460           480           500
TGCATTGTCTACCACCTGGAGAGCAAGATCTCCAAGCAAATCAGCTTCCTGATTATTGGC
 C  I  V  Y  H  L  E  S  K  I  S  K  Q  I  S  F  L  I  I  G

                520           540           560
CTGGCGTGGGGTGTCAGCGCTCTGCTGGCAAGTCCCCTTGCCATCTTCCGGGAGTACTCA
 L  A  W  G  V  S  A  L  L  A  S  P  L  A  I  F  R  E  Y  S

                580           600           620
CTGATTGAGATTATTCCTGACTTTGAGATTGTAGCCTGTACTGAGAAATGGCCCGGGGAG
 L  I  E  I  I  P  D  F  E  I  V  A  C  T  E  K  W  P  G  E

                640           660           680
GAGAAGAGTGTGTACGGTACAGTCTACAGCCTTTCCACCCTGCTAATCCTTCTACGTTTG
 E  K  S  V  Y  G  T  V  Y  S  L  S  T  L  L  I  L  L  Y  V  L
```

FIGURE 8D

```
        700       720       740
CCTCTGGGCATCATATCTTTCTCCTACACCCGGATCTCTGGAGTAAGCTAAAGAACCACGTT
 P  L  G  I  I  S  F  S  Y  T  R  I  W  S  K  L  K  N  H  V

        760       780       800
AGTCCTGGAGCTGCAAGTGACCATTACCATCAGCGAAGGCACAAAACGACCAAAATGCTC
 S  P  G  A  A  S  D  H  Y  H  Q  R  R  H  K  T  T  K  M  L

        820       840       860
GTGTGCCTGGTAGTGGTGTTTGCAGTCAGCTGGTTGCCCCTCCATGCCTTCCAACTTGCT
 V  C  V  V  V  F  A  V  S  W  L  P  L  H  A  F  Q  L  A

        880       900       920
GTGGACATCGACAGCCATGTCCTGGACCTGAAGGAGTACAAACTCATCTTCACCGTGTTC
 V  D  I  D  S  H  V  L  D  L  K  E  Y  K  L  I  F  T  V  F

        940       960       980
CACATTATTGCGATGTGCTCCACCTTCGCCAACCCCCTTCTCTATGGCTGGATGAACAGC
 H  I  I  A  M  C  S  T  F  A  N  P  L  L  Y  G  W  M  N  S
```

FIGURE 8E

```
1000            1020            1040
 .               .               .
AACTACAGAAAAGCTTTCCTCTCCAGCCTTCCGCTGTGAGCCAGAGGTTGGATGCCATTCAC
 N  Y  R  K  A  F  L  S  A  F  R  C  E  Q  R  L  D  A  I  H

1060            1080            1100
 .               .               .
TCGGAGGTGTCCATGACCTTCAAGGCTAAAAAGAAGAACCTGGAAGTCAAAAAGAACAATGGC
 S  E  V  S  M  T  F  K  A  K  K  K  N  L  E  V  K  K  N  N  G

1120            1140            1160
 .               .               .
CTCACTGACTCTTTTTCAGAGGCCACCAACGTGTAAGAATGCTGTGAAGTACGTGGGTA
 L  T  D  S  F  S  E  A  T  N  V  *

1180            1200            1220
 .               .               .
AATTGGCGACCACAGAGTTGCCAACCTGGTTAGGGAAGGTTTTCTGGCTAGTGCATGCCACCT

1240            1260            1280
 .               .               .
CCCATTGTATTGACCCTAAAAGCATCAGAGTGGAAGCCCCCAGCGGTATTGTTCCTGGAAA

1300            1320            1340
 .               .               .
ACTGGCTGGAAGAATGAGGAGGAGAAAATAAACAGATTGCTGTGGGCGCAACGTTCTGAT
```

FIGURE 9A

FIGURE 9A
FIGURE 9B
FIGURE 9C
FIGURE 9D

```
                                                    1
                -30           -10                   •
-50                                          MG
•
TTTCTGTTATAGATTCTTGTGCTATCTGCAGGCCAAATTGGAACTGAGGTGAAGATGGGC

      10           30           50
      •            •            •
CCATTAGGTGCAGAGGCCAGATGAGAATCAAAACTGTAGAAGTGGAATTCTATGGG
P L G A E A D E N Q T V E V K V E F Y G

      70           90           110
      •            •            •
TCGGGGCCCCACCACTCCCTAGAGGTGAGTTGCCCCCCCTGATCCCAGAGCCGGAGCTCATAGAC
S G P T T P R G E L P P D P E P E L I D

      130          150          170
      •            •            •
AGCACCAAACTGGTTGAGGTGCAGGTGCCTTATACTGGCCTATTGTTCCATCATCTTG
S T K L V E V Q V L I L A Y C S I I L

      190          210          230
      •            •            •
CTGGGGGTAGTTGGCAACTCTCTGGTAATCCATGTGGTGATCAAATTCAAGAGCATGCGC
L G V V G N S L V I H V V I K F K S M R
```

FIGURE 9B

250   270   290

ACAGTAACCAACTTTTTTATTGCCAACCTGGCTGTGGCGGATCTTTTGGTGAACACCCTG

T V T N F F I A N L A V A D L L V N T L

310   330   350

TGCCTGCCATTCACTCTTACCTATACCTTGATGGGGGAGTGGAAAATGGGTCCAGTTTTG

C L P F T L T Y T L M G E W K M G P V L

370   390   410

TGCCATTTGGTGCCCTATGCCCAGGGTCTGGCAGTACAAGTGTCCACAATAACTTTGACA

C H L V P Y A Q G L A V Q V S T I T L T

430   450   470

GTCATTGCTTTGGACCGACATCGTTGCATTGTCTACCACCTGGAGAGCAAGATCTCCAAG

V I A L D R H R C I V Y H L E S K I S K

490   510   530

CAAATCAGCTTCCTGATTATTGGCGTGGGGGTGTCAGCGCCTGCTGGCAAGTCCC

Q I S F L I I G L A W G V S A L L A S P

FIGURE 9C

```
       550         570         590
CTTGCCATCTTCCGGGAGTACTCACTGATTGAGATTATTCCTGACTTTGAGATTGTAGCC
 L  A  I  F  R  E  Y  S  L  I  E  I  I  P  D  F  E  I  V  A

       610         630         650
TGTACTGAGAAATGGCCCGGGGAGGAGAAGAGTGTGTACGGTACAGTCTACAGCCTTTCC
 C  T  E  K  W  P  G  E  E  K  S  V  Y  G  T  V  Y  S  L  S

       670         690         710
ACCCTGCTAATCCTCTACGTTTTGCCTCTGGGCATCATATCTTTCTCCTACACCCGGATC
 T  L  L  I  L  Y  V  L  P  L  G  I  I  S  F  S  Y  T  R  I

       730         750         770
TGGAGTAAGCTAAAGAACAACCACGTTAGTCCTGGAGCTGCAAGTGACCATTACCATCAGCGA
 W  S  K  L  K  N  H  V  S  P  G  A  A  S  D  H  Y  H  Q  R

       790         810         830
AGGCACAAAATGACCAAAATGCTCGTGTGCGTGGTAGTGGTGTTTGCAGTCAGCTGGCTG
 R  H  K  M  T  K  M  L  V  C  V  V  V  V  F  A  V  S  W  L
```

FIGURE 9D

850
CCCCTCCATGCCTTCCAACTTGCTGTGGACATCGACAGCCATGTCCTGGACCTGAAGGAG
P L H A F Q L A V D I D S H V L D L K E

870   890

910
TACAAACTCATCTTCACCGTGTTCCACATTATTGCGATGTGCTCCACCTTCGCCAACCCC
Y K L I F T V F H I I A M C S T F A N P

930   950

970
CTTCTCTATGGCTGGATGAACAGCAACTACAGAAAAGCTTTCCTCTCAGCCTTCCGCTGT
L L Y G W M N S N Y R K A F L S A F R C

990   1010

1030
GAGCAGAGGTTGGATGCCATTCACTCGGAGGTGTCCATGACCTTCAAGGCTAAAAAGAAC
E Q R L D A I H S E V S M T F K A K K N

1050   1070

1090
CTGGAAGTCAAAAAGAACAATGGCCTCACTGACTCTTTTCAGAGGCCACCAACGTGTAA
L E V K K N N G L T D S F S E A T N V *

1110   1130

FIGURE 10A

FIGURE 10A
FIGURE 10B
FIGURE 10C
FIGURE 10D

```
Hum Y2   ATGGGTTCCAATAGGTGCAGAGGCTGATGAGAACCAGACAGTGGAAGAAAT   50
Rat Y2a  ATGGGCCCATTAGGTGCAGAGGCAGATGAGAATCAAAACTGTAGAAGTGAA   50
Rat Y2b  ATGGGCCCATTAGGTGCAGAGGCAGATGAGAATCAAACTGTAGAAGTGAA    50

Hum Y2   GAAGGTGGAACAATACGGGCCCACAAACAACTCCTAGAGGTGAACTGGTCC   100
Rat Y2a  AGTGGAACTCTATGGGTCGGGGCCCCACCACTCCTAGAGGTGAGTTGCCCC   100
Rat Y2b  AGTGGAATTCTATGGGTCGGGGCCCCACCACTCCTAGAGGTGAGTTGCCCC·  100

Hum Y2   CTGACCCTGAGCCAGAGCTTATAGATAGTACCAAGCTGATTGAGGTACAA   150
Rat Y2a  CTGATCCAGAGCCGGGAGCTCATAGACACAGCACCAAACTGGTTGAGGTGCAG   150
Rat Y2b  CTGATCCAGAGCCGGGAGCTCATAGACACAGCACCAAACTGGTTGAGGTGCAG   150

Hum Y2   GTTGTTCTCATATTGGCCTACTGCTCCATCATCTTGCTTGGGGTAATTGG   200
Rat Y2a  GTGGTCCTTATACTGGCCTATTGTTCCATCATCTTGCTTGCTGGGCGTAGTTGG   200
Rat Y2b  GTGGTCCTTATACTGGCCTATTGTTCCATCATCTTGCTTGCTGGGCGTAGTTGG   200

Hum Y2   CAACTCCTTGGTGATCCATGTGGTGATCAAATTCAAGAGCACATGCGCCACAG   250
Rat Y2a  CAACTCTCTGGTAATCCATGTGGTGATCAAATTCAAGAGCACATGCGCGCCACAG   250
Rat Y2b  CAACTCTCTGGTAATCCATGTGGTGATCAAATTCAAGAGCACATGCGCGCCACAG   250

Hum Y2   TAACCAACTTTTTCATTGCCAATCTGGCTGTGGCCGAGATCTTTGGTGAAC   300
Rat Y2a  TAACCAACTTTTTATTGCCAACCTGGCTGTGGCCGGATCTTTGGTGAAC   300
Rat Y2b  TAACCAACTTTTTATTGCCAACCTGGCTGTGGCCGGATCTTTGGTGAAC   300

Hum Y2   ACTCTGTGTCTACCGTTCACTCTTCTTACCTTAATGGGGAGTGGAA   350
Rat Y2a  ACCCTGTGCCTGCCATTCACTCTTCTTATACCTTGATGGGGAGTGGAA   350
Rat Y2b  ACCCTGTGCCTGCCATTCACTCTTCTTATACCTTGATGGGGAGTGGAA   350
```

FIGURE 10B

```
Hum Y2   AATGGGTCCTGTCCTGTGCCACCTGGTGCCCTGGTGCCCTATGCCCCAGGGCCTGGCAG  400
Rat Y2a  AATGGGTCCAGTTTGTGCCATTTGGTGCCCTATGCCCTATGCCCCAGGGTCTGGCAG    400
Rat Y2b  AATGGGTCCAGTTTGTGCCATTTGGTGCCCTATGCCCTATGCCCCAGGGTCTGGCAG    400

Hum Y2   TACAAGTATCCACAATCACCTTGACAGTAATTGCCCTGGACCGGCACAGG          450
Rat Y2a  TACAAGTGTCCACAATAACTTTGACAGTCATTGCTTTGGACCGACATCGT         450
Rat Y2b  TACAAGTGTCCACAATAACTTTGACAGTCATTGCTTTGGACCGACATCGT         450

Hum Y2   TGCATCGTCTACCACCTAGAGAGCAAGATCTCCAAGCGGAATCAGCTTCCT        500
Rat Y2a  TGCATTGTCTACCACCTGGAGAGCAAGATCTCCAAGCAAATCAGCTTCCT         500
Rat Y2b  TGCATTGTCTACCACCTGGAGAGCAAGATCTCCAAGCAAATCAGCTTCCT         500

Hum Y2   GATTATTGGCTTGGCCTGGCGTGGGGCATCAGTGCCCTGCTGCAAGTCCCCCTGG     550
Rat Y2a  GATTATTGGCCTGGCGTGGGGTGTCAGCGCGTCAGCCGGCTGCTGGCAAGTCCCCCTTG  550
Rat Y2b  GATTATTGGCCTGGCGTGGGGTGTCAGCGCGTCAGCCGGCTGCTGGCAAGTCCCCCTTG  550

Hum Y2   CCATCTTCCGGGAGTATTCGCTGATTGAGATCATCCCGGACTTTGAGATT        600
Rat Y2a  CCATCTTCCGGGAGTACTCACTGATTGAGATTATTCCTGACTTTGAGATT         600
Rat Y2b  CCATCTTCCGGGAGTACTCACTGATTGAGATTATTCCTGACTTTGAGATT         600

Hum Y2   GTGGCCTGTACTGAAAAGTGGCCTGGCCGGGGAGAGAGCATCTATGGCAC         650
Rat Y2a  GTAGCCTGTACTGAGAAATGGCCCGGGGGAGAGAGAGTGTACGGTAC            650
Rat Y2b  GTAGCCTGTACTGAGAAATGGCCCGGGGGAGAGAGAGTGTACGGTAC            650

Hum Y2   TGTCTATAGTCTTTCTTCCTTGTTGATCTTGTATGTTTTGCCTCTGGGCA         700
Rat Y2a  AGTCTACACAGCCTTTCCACCCTGCTAATCCCTGCGTTTCTACGTTTGCCTCTGGGCA  700
Rat Y2b  AGTCTACACAGCCTTTCCACCCTGCTAATCCCTGCGTTTCTACGTTTGCCTCTGGGCA  700
```

FIGURE 10C

```
Hum Y2   TTATATCATTTCCTACACTCGCATTGGAGTAAATTGAAGAACCATGTC      750
Rat Y2a  TCATATCTTTCTCCTACACCCGGATCTGGAGTAAGCTAAAGAACCACGTT    750
Rat Y2b  TCATATCTTTCTCCTACACCCGGATCTGGAGTAAGCTAAAGAACCACGTT    750

Hum Y2   AGTCCTGGAGCTGCAAATGACCACTACCATCAGCGAAGGCAAAAAACCAC    800
Rat Y2a  AGTCCTGGAGCTGCAAGTGACCATTACCATCAGCGAAGGCACAAAAGGAC    800
Rat Y2b  AGTCCTGGAGCTGCAAGTGACCATTACCATCAGCGAAGGCACAAAATGAC    800

Hum Y2   CAAAATGCTGGTGTGTGGTGGTGTTGCGGTGGTCAGCTGGCTGCCTC      850
Rat Y2a  CAAAATGCTCGTGTGCGTGGTGGTGGTGTTTGCAGTCAGCTGGCTGCCCC    850
Rat Y2b  CAAAATGCTCGTGTGCGTGGTGGTGGTGTTTGCAGTCAGCTGGCTGCCCC    850

Hum Y2   TCCATGCCTTCCAGCTTGCCGTTGACATTGACAGCCAGGTCCTGGACCTG    900
Rat Y2a  TCCATGCCTTCCAACTTGCTGTGGACATCGACAGCCATGTCCTGGACCTG    900
Rat Y2b  TCCATGCCTTCCAACTTGCTGTGGACATCGACAGCCATGTCCTGGACCTG    900

Hum Y2   AAGGAGTACAAACTCATCTTCACAGTGTTCCACATCATCGCCATGTGCTC    950
Rat Y2a  AAGGAGTACAAACTCATCTTCACCGTGTTCCACATTATTGCGATGTGCTC    950
Rat Y2b  AAGGAGTACAAACTCATCTTCACCGTGTTCCACATTATTGCGATGTGCTC    950

Hum Y2   CACTTTGCCAATCCCCTTCTCTATGGCTGGATGAACAGCAACTACAGAA    1000
Rat Y2a  CACCCTTCGCCAACCCCCTTCTCTATGGCTGGATGAACAGCAACTACAGAA  1000
Rat Y2b  CACCCTTCGCCAACCCCCTTCTCTATGGCTGGATGAACAGCAACTACAGAA  1000

Hum Y2   AGGCTTTCCTCTCGGCCTTCCGCTGTGAGCAGCGGTTGGATGCCATTCAC   1050
Rat Y2a  AAGCTTTCCTCTCAGCCTTCCGCTGTGAGCAGCAGGTTGGATGCCATTCAC  1050
Rat Y2b  AAGCTTTCCTCTCAGCCTTCCGCTGTGAGCAGCAGGTTGGATGCCATTCAC  1050
```

FIGURE 10D

```
Hum Y2   TCTGAGGTGTCCGTGACATTCAAGGCTAAAAAGAACCTGGAGGTCAGAAAA  1100
Rat Y2a  TCGGAGGTGTCCATGACCTTCAAGGCTAAAAAGAACCTGGAAGTCAAAAA   1100
Rat Y2b  TCGGAGGTGTCCATGACCTTCAAGGCTAAAAAGAACCTGGAAGTCAAAAA   1100

Hum Y2   GAACAGTGGCCCCCAATGACTCTTTCACAGAGGCTACCAATGTCTAA      1146
Rat Y2a  GAACAATGGCCCTCACTGACTCTTTTCAGAGGCCACCAACGTGTAA      1146
Rat Y2b  GAACAATGGCCCTCACTGACTCTTTTCAGAGGCCACCAACGTGTAA      1146
```

FIGURE 11A

FIGURE 11A
FIGURE 11B

```
Hum Y2   MGPIGAEADENQTVEEMKVEQYGP.QTTPRGELVPDDPEPELIDSTKLIEV   49
Rat Y2a  MGPLGAEADENQTV.EVKVELYGSGPTTPRGELPPDPEPELIDSTKLVEV   49
Rat Y2b  MGPLGAEADENQTV.EVKVELYGSGPTTPRGELPPDPEPELIDSTKLVEV   49
                                                         II
                       I                                ___
Hum Y2   QVVLILAYCSIILLGVIGNSLVIHVVI  KFKSMRTVTN  FFIANLAVADLLV   99
Rat Y2a  QVVLILAYCSIILLGVVGNSLVIHVVI  KFKSMRTVTN  FFIANLAVADLLV   99
Rat Y2b  QVVLILAYCSIILLGVVGNSLVIHVVI  KFKSMRTVTN  FFIANLAVADLLV   99
                                                       III
                                                       ___
Hum Y2   NTLCLPFTLT  YTLMGEWKMGPVLCH  LVPYAQGLAVQVSTITLTVIAL  DRH  149
Rat Y2a  NTLCLPFTLT  YTLMGEWKMGPVLCH  LVPYAQGLAVQVSTITLTVIAL  DRH  149
Rat Y2b  NTLCLPFTLT  YTLMGEWKMGPVLCH  LVPYAQGLAVQVSTITLTVIAL  DRH  149
                              IV
                              ___
Hum Y2   RCIVYHLESKISKRI  SFLIIGLAWGISALLASPLAIF  REYSLIEIIPDFE   199
Rat Y2a  RCIVYHLESKISKQI  SFLIIGLAWGVSALLASPLAIF  REYSLIEIIPDFE   199
Rat Y2b  RCIVYHLESKISKQI  SFLIIGLAWGVSALLASPLAIF  REYSLIEIIPDFE   199
                   V
                   ___
Hum Y2   IVACTEKWPGEEKSIYGT  VYSLSSLLILYVLPLGIISFSYT  RIWSKLKNH   249
Rat Y2a  IVACTEKWPGEEKSVYGT  VYSLSTLLILYVLPLGIISFSYT  RIWSKLKNH   249
Rat Y2b  IVACTEKWPGEEKSVYGT  VYSLSTLLILYVLPLGIISFSYT  RIWSKLKNH   249
                                VI
                                ___
Hum Y2   VSPGAANDHYHQRRQKTTKM  LVCVVVFAVSWLPLHAFQLAVDI  DSQVLD   299
Rat Y2a  VSPGAASDHYHQRRHKTTKM  LVCVVVFAVSWLPLHAFQLAVDI  DSHVLD   299
Rat Y2b  VSPGAASDHYHQRRHKMTKM  LVCVVVFAVSWLPLHAFQLAVDI  DSHVLD   299
```

FIGURE 11B

```
                            VII
Hum Y2    LKEYKL  IFTVFHIIAMCSTFANPLLYGWM NSNYRKAFLSAFRCEQRLDAI    349
Rat Y2a   LKEYKL  IFTVFHIIAMCSTFANPLLYGWM NSNYRKAFLSAFRCEQRLDAI    349
Rat Y2b   LKEYKL  IFTVFHIIAMCSTFANPLLYGWM NSNYRKAFLSAFRCEQRLDAI    349

Hum Y2    HSEVSVTFKAKKNLEVRKNSGPNDSFTEATNV    381
Rat Y2a   HSEVSMTFKAKKNLEVKKNNGLTDSFSEATNV    381
Rat Y2b   HSEVSMTFKAKKNLEVKKNNGLTDSFSEATNV    381
```
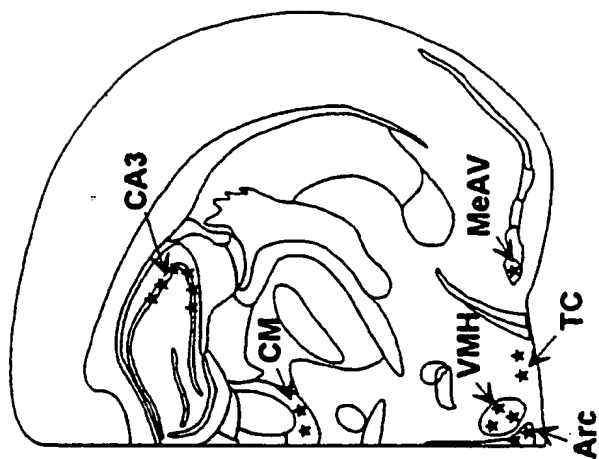
FIGURE 12A

FIGURE 12B

FIGURE 12C
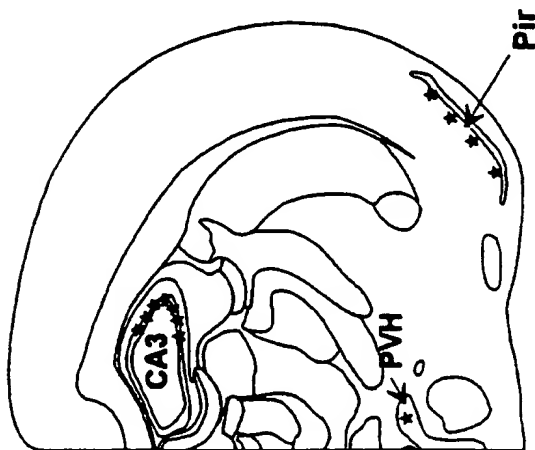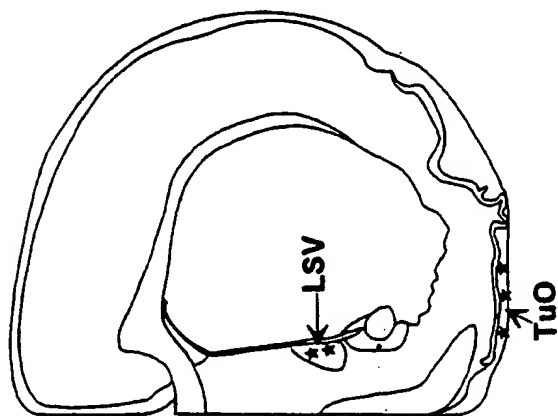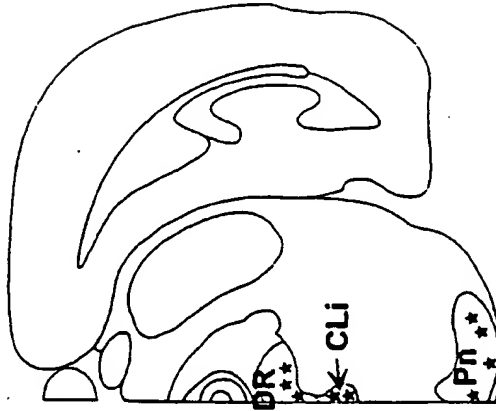
FIGURE 12F

FIGURE 12E
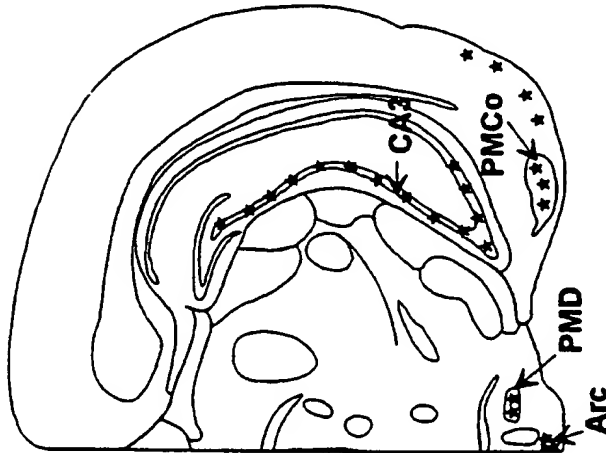
FIGURE 12D
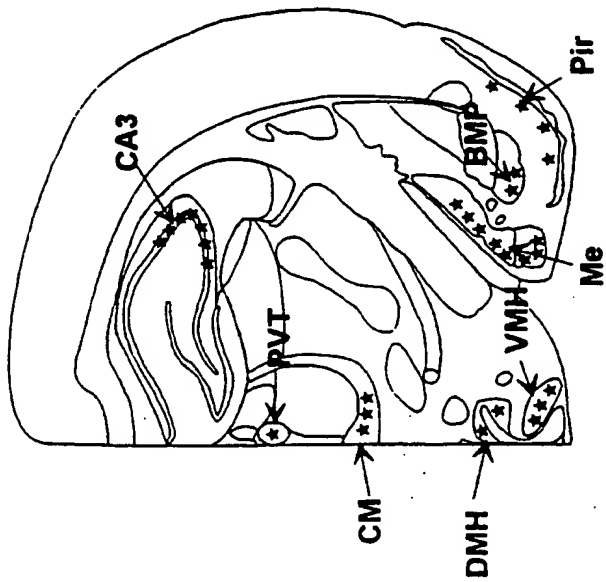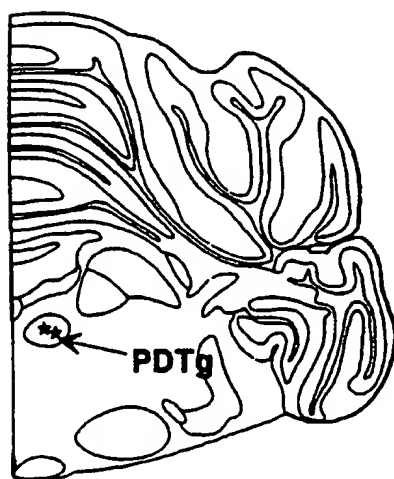
FIGURE 12G

FIGURE 12H
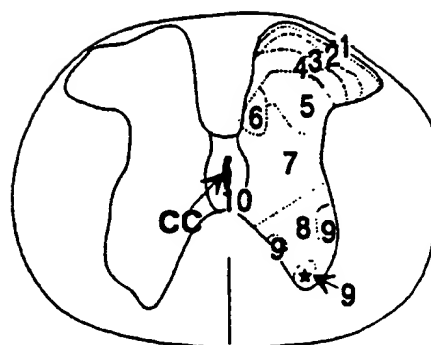
Cervical Spinal Cord
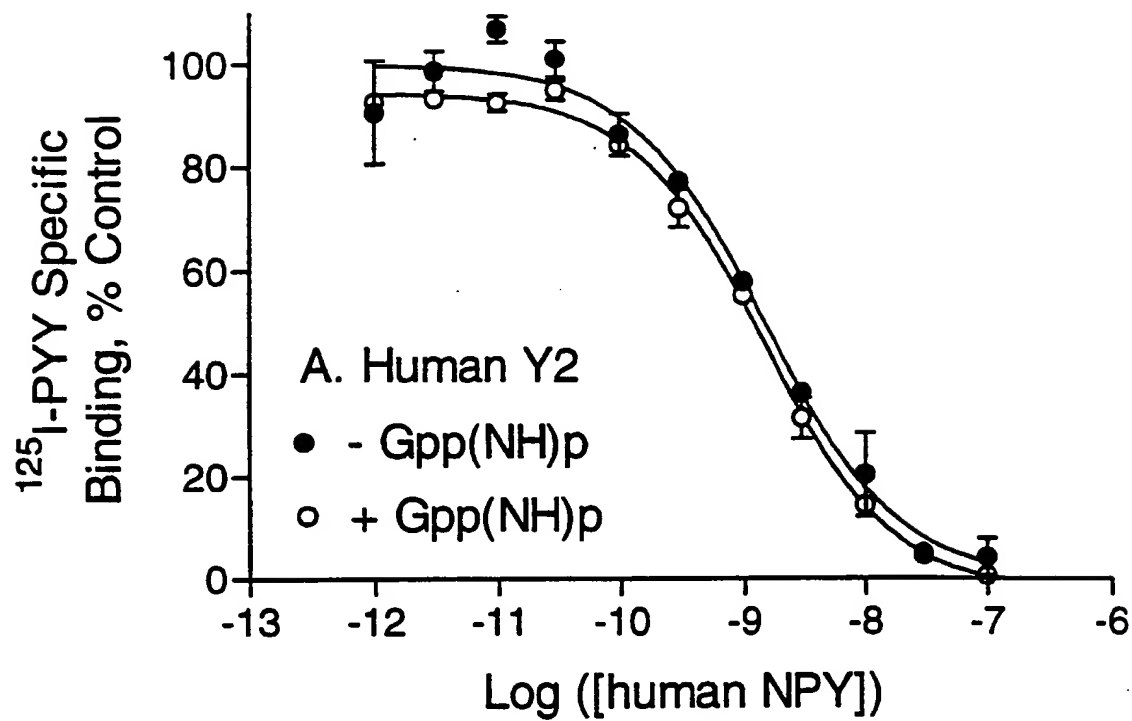
FIGURE 13A

FIGURE 13B
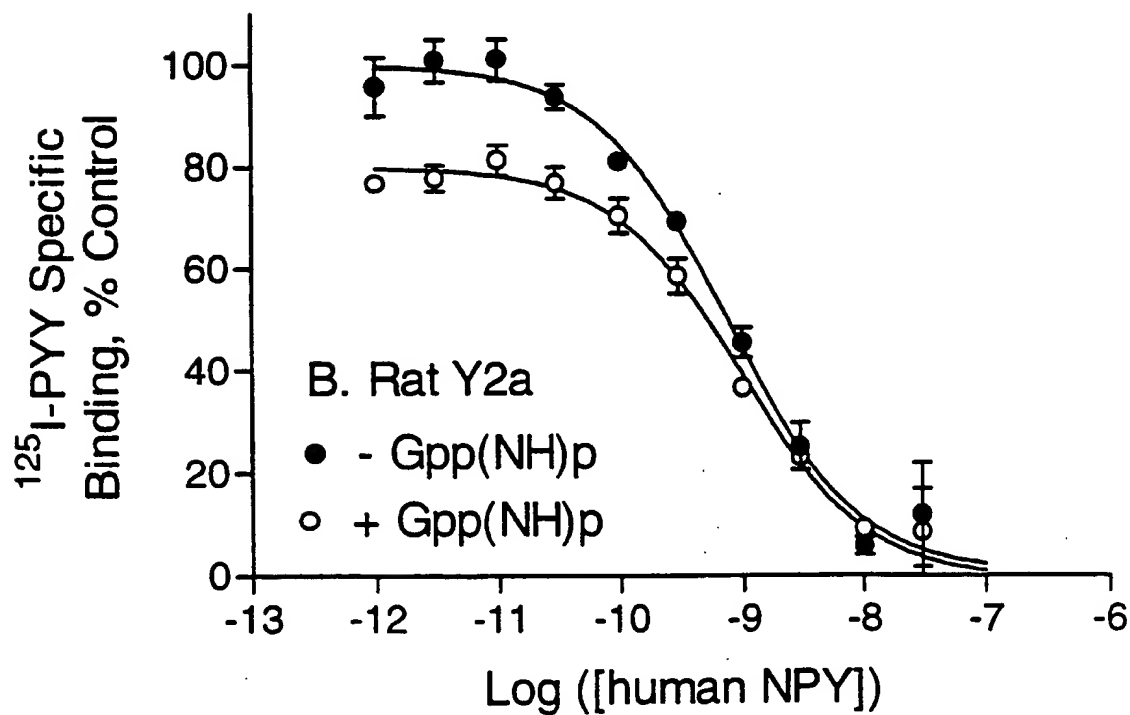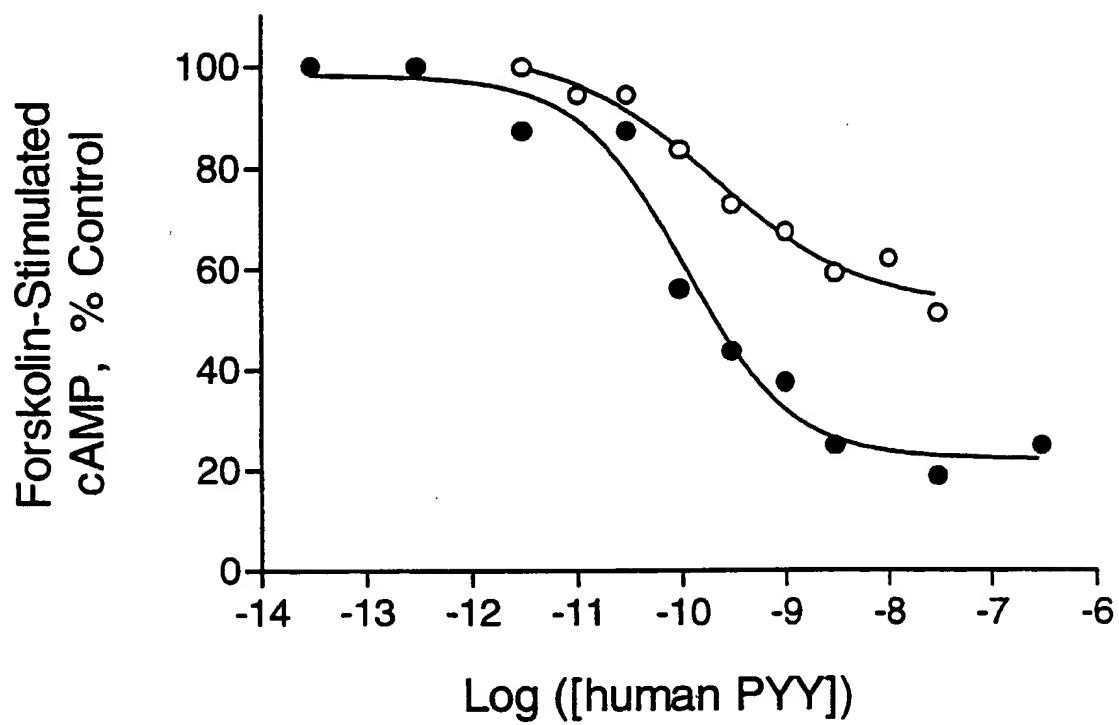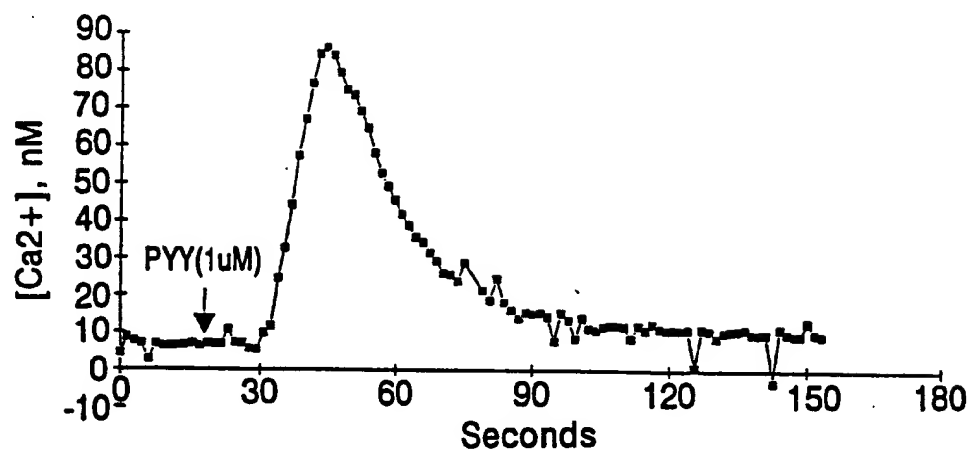
FIGURE 14

FIGURE 15A

FIGURE 15B
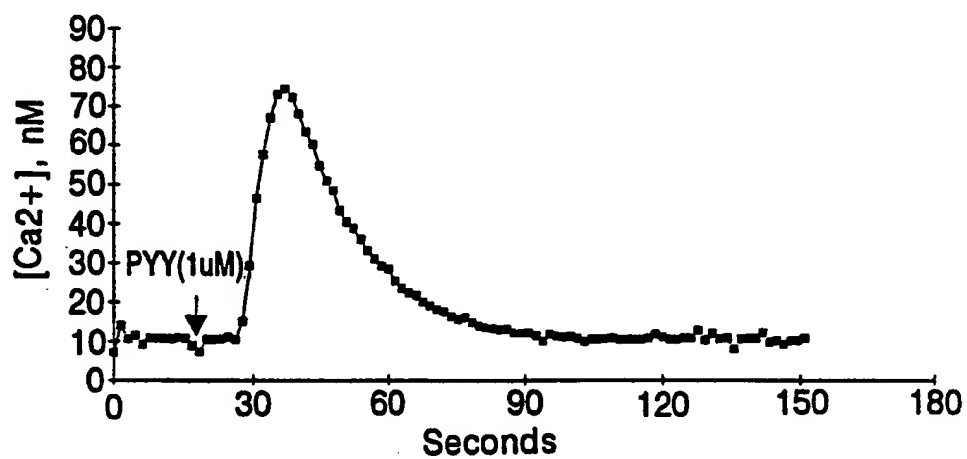
FIGURE 15C
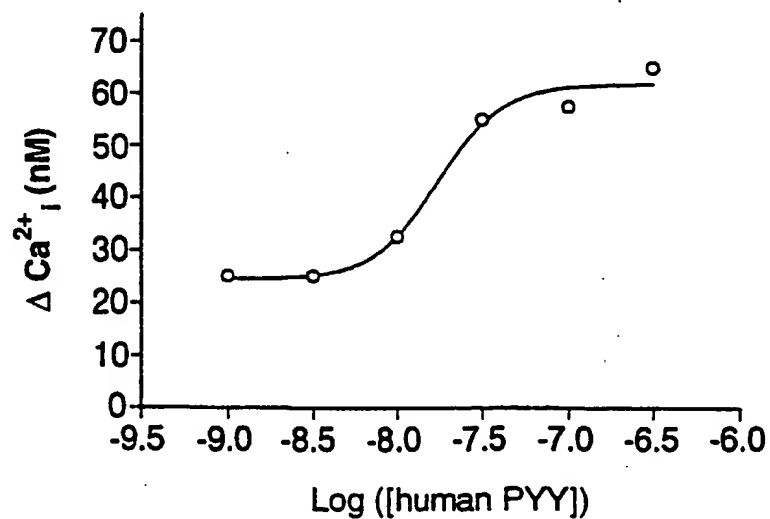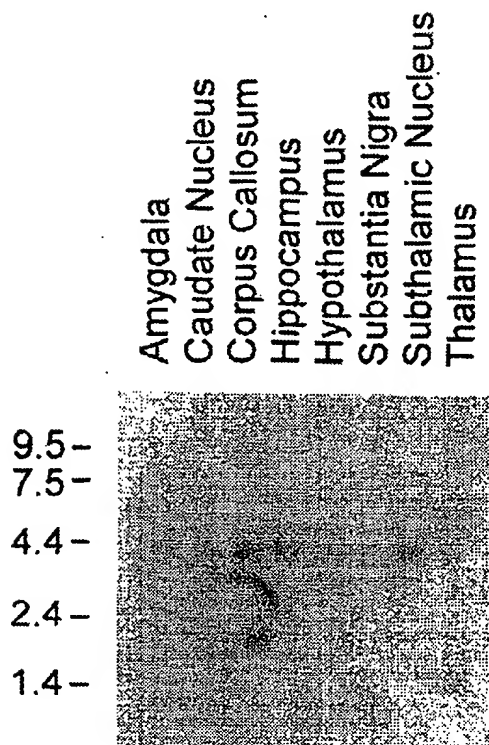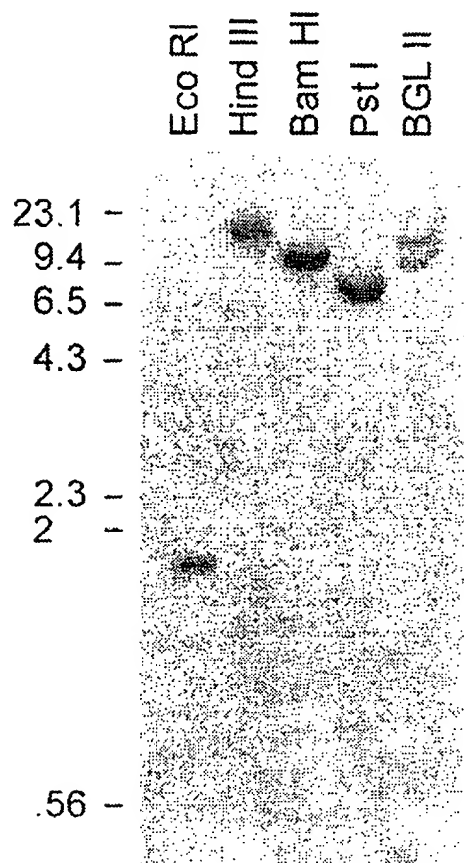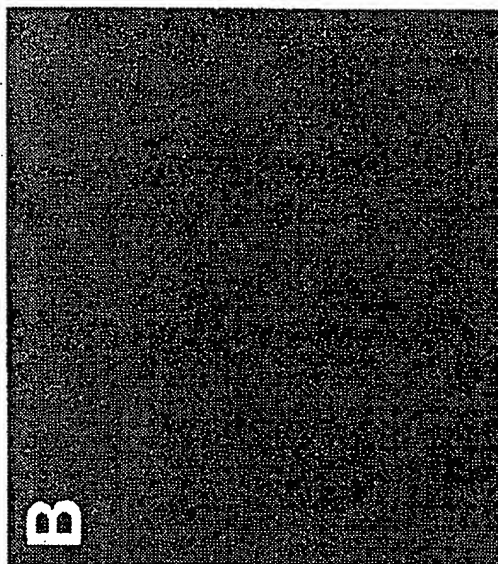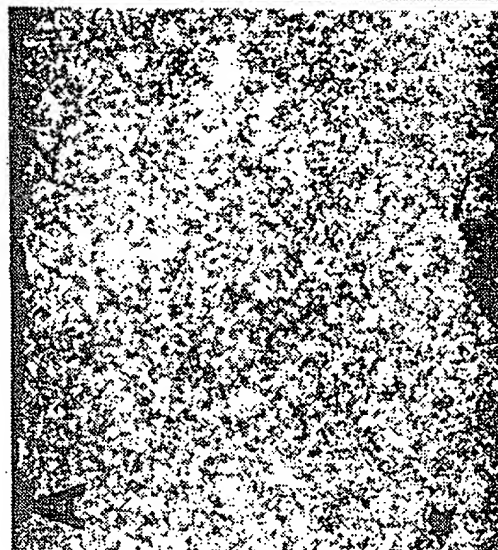
FIGURE 16

FIGURE 17

FIGURE 18A

FIGURE 18B

FIGURE 18C

FIGURE 18D
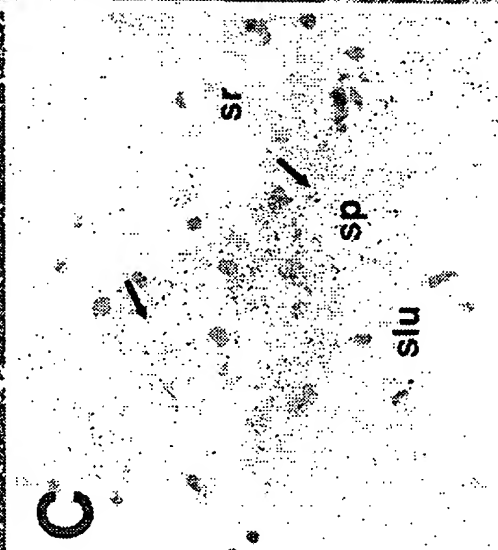
INTERNATIONAL SEARCH REPORT

A.	CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   435/69.1, 70.1, 70.3, 71.1, 172.1, 240.1, 240.2, 320.1; 536/23.1, 23.5, 24.31; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS; STN files Biosis, Medline, EMBASE, CA, WPIDS; search terms include: neuropeptide Y, NPY, Y1, Y2 (all with receptor#); sequence search.

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Annals of the New York Academy of Sciences, Volume 611, issued 15 November 1990, A. Inui et al, "Peptide YY Receptors in Mammalian and Avian Brains", pages 350-352, see entire document.	1-42, 122, 156
Y	Neuroscience Letters, Volume 140, issued 1992, J. Narvaez et al, "Intracerebroventricularly Administered Pertussis Toxin Blocks the Central Vasopressor Action of Neuropeptide Y(13-36) in the Awake Unrestrained Male Rat", pages 273-276, see entire document.	1-42, 122, 156

[X]	Further documents are listed in the continuation of Box C.	[ ]	See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 APRIL 1995	16 MAY 1995

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	ARIE M. MICHELSOHN, PH.D.
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Form PCT/ISA/210 (second sheet)(July 1992)★

International application No.

PCT/US95/01469

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Brain Research, Volume 596, issued 1992, C. Blasquez et al, "Neuropeptide Y Inhibits α-MSH Release from Rat Hypothalamic Slices Through a Pertussis Toxin-sensitive G Protein", pages 163-168, see entire document.	1-42, 122, 156
Y	Life Sciences, Volume 50, issued 1991, C. Wahlestedt et al, "Identification of Cultured Cells Selectively Expressing Y1-, Y2-, or Y3-Type Receptors for Neuropeptide Y/Peptide YY", pages PL-7-PL-12, see entire document.	1-42, 122, 156
Y	Journal of Biological Chemistry, Volume 266, Number 35, issued 15 December 1991, S. Sheikh et al, "Solubilization and Affinity Purification of the Y_2 Receptor for Neuropeptide Y and Peptide YY from Rabbit Kidney", pages 23959-23966, see entire document.	1-42, 122, 156
Y	Annals of the New York Academy of Sciences, Volume 611, issued 15 November 1990, C. Wahlestedt et al, "Neuropeptide Y Receptor Subtypes, Y1 and Y2", pages 7-25, see pages 12-16 and 19-21.	1-42, 122, 156

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01469

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-42, 122 and 156

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12N 5/10, 5/16, 7/01, 15/00, 15/09, 15/10, 15/11, 15/12, 15/16, 15/63; C07H 21/00, 21/04; C07K 14/00, 14/705, 14/72

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 70.1, 70.3, 71.1, 172.1, 240.1, 240.2, 320.1; 536/23.1, 23.5, 24.31; 530/350

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-42, 122 and 156, drawn to nucleic acids, probes and corresponding proteins, classified in U.S. Class 435, subclass 69.1, for example.

Group II, claims 43-45 and 51-55, drawn to antisense oligonucleotides, classified in U.S. Class 536, subclass 23.1; Class 514, subclass 44.

Group III, claims 46-50 and 56, drawn to antibodies, classified in U.S. Class 530, subclass 387.1.

Group IV, claims 57-62, drawn to transgenic animals, classified in U.S. Class 800, subclass 2.

Group V, claims 63-72, 74-87, 89-90, 98-115 and 117-118, drawn to binding assays, classified in U.S. Class 436, subclass 501.

Group VI, claims 73, 92 and 93, drawn to ligands, classified in U.S. Class 532, subclass 1, for example.

Group VII, claims 88, 91, 116 and 119, drawn to cells, classified in U.S. Class 435, subclass 240.1.

Group VIII, claims 94-95, 151 and 152, drawn to agonists, classified in U.S. Class 514, subclass 1.

Group IX, claims 96-97, 147 and 148, drawn to antagonists, classified in U.S. Class 514, subclass 1.

Group X, claims 120-121, drawn to drugs, classified in U.S. Class 514, subclass 1.

Group XI, claims 123-124, and 153, drawn to methods of treatment with agonists, classified in U.S. Class 514, subclass 1.

Group XII, claims 125-126, 149 and/or 127-135, drawn to methods of treatment with antagonists, classified in U.S. Class 514, subclass 1.

Group XIII, claim 136 and/or claims 138-142, drawn to methods of treatment with antibodies, classified in U.S. Class 424, subclass 85.8.

Group XIV, claim 137 and/or claims 138-142, drawn to methods of treatment with antisense oligonucleotides, classified in U.S. Class 514, subclass 44.

Group XV, claim 143, drawn to method of detection using antibodies, classified in U.S. Class 436, subclass 501.

Group XVI, claims 144-145, drawn to methods of determining physiological effects using transgenic animals, classified in U.S. Class 424, subclass 9.

Group XVII, claims 154-155, drawn to methods of diagnosis using nucleic acid probes, classified in U.S. Class 435, subclass 6.

Group XVIII, claim 146, drawn to method for identifying antagonists using transgenic animals, classified in U.S. Class 424, subclass 9.

Group XIX, claim 150, drawn to method for identifying agonists using transgenic animals, classified in U.S. Class 424, subclass 9.

The inventions listed as Groups I-XIX do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features; i.e., the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept, for the following reasons:

Groups I-IV and VI-X are materially distinct compositions of matter that are distinguished, each from the other, by their special technical features:

The polynucleotides and polypeptides of Group I, the antisense oligonucleotides of Group II, the antibodies of Group III, the transgenic animals of Group IV, the ligands of Group VI, the cells of Group VII, the agonists of Group VIII, the antagonists of Group XIX, and the drugs of Group X have materially different structures and functions.

The compositions of Groups I-IV and VI-X are not restricted for use in the methods of Groups V and XI-XIX, but can be used for materially different purposes, or at least for a single method from among those listed in Groups V and XI-XIX.

Groups V and XI-XIX are materially distinct methods which are distinguished, each from the other, by their process steps. The process steps accomplish materially distinct purposes and these special technical features do not link the methods as to form a single inventive concept.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

Drugs; agonists; antagonists; antibodies; and antisense oligonucleotides.

The claims are deemed to correspond to the species listed above in the following manner:

Claim 124: method employing agonist; Claim 126: method employing antagonist; Claim 136: method employing antisense oligonucleotides; Claim 137: method employing antibodies.

The following claims are generic: 123 (claiming either of the species of drugs or agonists); 125 and 127-135 (claiming either of the species of drugs or antagonists); 138-142 (claiming either of the species of antibodies or antisense oligonucleotides).

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The drugs, agonists, antagonists, antibodies and antisense oligonucleotides are materially different compositions of matter that are distinguished, each from the other, by their special technical features, including their materially different structures and functions.

Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.